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Bachelor Thesis

"Optimization of the Use of Differences in FiO2 for the Study of Blood Perfusion in Magnetic Resonance Imaging"

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ABSTRACT

Brain tumors and ischemic diseases are one of the most aggressive causes of death [1]. The study of cerebral perfusion, which is the passage of arterial blood from the circulatory system to the tissues, plays a key role in the diagnosis of these threatening medical conditions. Currently, cerebral perfusion is commonly studied using magnetic resonance imaging (MRI), which is a non-ionizing image modality. Perfusion in MRI is assessed in different ways; Dynamic Susceptibility Contrast (DSC) is the more wide spread method. DSC consists on injecting a bolus of an external contrast in blood, which in most cases is gadolinium, a paramagnetic contrast agent that facilitates transversal and longitudinal relaxation of the surrounding tissue. Therefore, the MRI signal varies when the contrast goes through the vessels. These changes in signal can be seen in T_2/T_2^* weighted images, where the pass of gadolinium decreases the received signal. The shape of the intensity curves behaves differently in pathogenic tissue.

The main disadvantage of this procedure is precisely the use of this external contrast. It involves higher cost, discomfort and health risks for patients. Furthermore, this method does not rely on the intrinsic properties of blood inducing error in the measures. This project tries to solve this problem by using blood as an endogenous contrast for DSC. As Blood Oxygenated Level Dependent (BOLD) effect describes, deoxyhemoglobin is paramagnetic as gadolinium, so its higher concentration facilitates the transversal and longitudinal relaxation of spins. Therefore, decreasing the Fraction of Inspired Oxygen (FiO_2) of a subject for a brief period of time, may lead to similar results to those obtained with gadolinium experiments. This project studies the relationship between changes in FiO_2 and MRI signal. The MR images can be obtained using Echo Planar imaging (EPI) sequences which is a rapid imaging technique used in DSC. In this work the results obtained performing dynamic susceptibility contrast using changes in fraction of inspired oxygen, by forcing 100% nitrogen inspired gas (N_2-DSC), were compared with gadolinium experiments (Gd-DSC) in both healthy and stroke-induced rats. Besides, a MATLAB code was developed to generate the parametric maps of the perfusion parameters and to assess if comparable results between N_2-DSC and Gd-DSC were obtained.

ACRONYMS:

AIF: Arterial Input Function

ASL: Arterial Spin Labeling

BBB: Blood Brain Barrier

BOLD: Blood oxygenated level dependent

CBF: Cerebral Blood Flow

CBV: Cerebral Blood Volume

DCE: Delayed Contrast Enhancement

DSC: Dynamic Susceptibility Contrast

EPI: Echo planar imaging

$Fi0_2$: Fraction of Inspired Oxygen

Gd -DSC: Dynamic Susceptibility Contrast using gadolinium-based contrasts

MRI: Magnetic Resonance Imaging

MTT: Mean transit Time

N_2-DSC: Dynamic Susceptibility Contrast using changes in the fraction of inspired oxygen, substituting the inspired oxygen by nitrogen

NMR: Nuclear Magnetic Resonance

$SpO_{2:}$ Saturation of oxygen

TTP: Time to peak

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1. Introduction

Brain perfusion is the passage of blood through the brain's vascular network, closely related to the delivery of oxygen and nutrients to the tissue. Cerebral perfusion is widely studied using Magnetic Resonance Imaging [2], which is a non-ionizing image modality, an advantage over other imaging modalities, such as Nuclear Medicine and Computer Tomography which involve the harmful effects produced by ionizing radiation. Another benefit of MRI over the mentioned modalities is its flexibility allowing one to adapt the study to the area and physiological process of interest [3]. MR perfusion provides information of several health conditions such as ischemia, brain tumors and neurodegenerative diseases without perturbing the system of study. The data generated by image post-processing of perfusion studies can be used for diagnosis and for giving a personalized treatment to every patient.

1.1. Magnetic Resonance Imaging

Magnetic Resonance Imaging is a tomographic imaging modality. It is a powerful tool for evaluation of brain anatomy as it provides especially good contrast and spatial resolution in soft tissue. Its application is not only limited to structural imaging; it has recently expanded to the functional evaluation of brain, assessing different parameters such as diffusion or perfusion. Due to the development of fast imaging techniques, it is possible to study the function of the brain with a temporal resolution close to that of the physiological processes that occur in the brain.

1.1.1. Physical principles

Magnetic Resonance Imaging is based on Nuclear Magnetic Resonance phenomenon (NMR), which was discovered in the first half of the XX century [4]. Nuclear Magnetic Resonance is a complex process that cannot be understood making solely use of classical physics, being necessary the use of quantum mechanics to explain this phenomenon. Only nuclei that possess a non-zero spin experiment the NMR phenomenon. Spin is a fundamental property that elementary particles have; however, it is not as intuitive as mass or electric charge. Spin interacts with the magnetic field in the same way as angular momentum interacts with the gravitational field. Therefore, NMR is based on the interaction between a nucleus with a non-zero spin and a magnetic field. Nuclei with +-1/2 spin are the most commonly studied, because they have higher resolution. Several atoms present in our body (1H, 31P, 23Na) fit this category. However, 1H is by a great deal the most abundant in the body and the most sensitive stable isotope; therefore, imaging dependent on hydrogen is the most widespread technique [5].

Under the influence of a magnetic field B_0 a sample shows a net magnetization vector aligned with the field (Figure 1). This vector is the consequence of the magnetic moments of each individual nucleus. Under the influence of B_0 the spins of the nuclei precess around B_0 axis at a certain frequency called Larmor frequency (w_0), which depends on the magnitude of the magnetic field and the nature of the atom, in this case hydrogen.

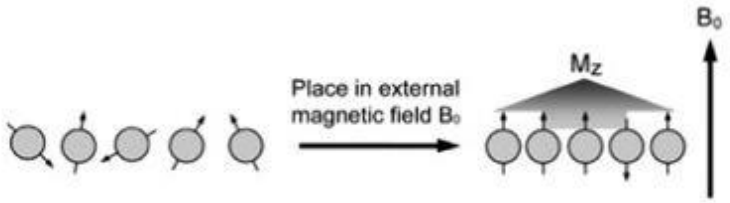


Figure 1 Net magnetization vector under the influence of a constant magnetic field (B_0) [3].

If a radiofrequency pulse (RF) of a frequency equal to the Larmor frequency of the nuclei is applied to the sample, it is absorbed (resonance phenomenon) modifying the equilibrium state of the net magnetization and causing the magnetization vector to flip down to the transversal plane. Depending on the intensity of RF and the time it has been applied, the net magnetization vector will flip towards the transversal plane certain amount of degrees.

After excitation the magnetic vector tends to recover its equilibrium. The recoveries of the longitudinal component and of the transversal component are two independent processes that depend on different properties and occur at different rates.

Longitudinal relaxation

Longitudinal relaxation is the recovery of the longitudinal component of the magnetic moment. It is also known as T_1 relaxation or spin-lattice relaxation. The extra energy of the nuclei is transferred to the lattice in form of heat. This process is characterized by T_1 value which is defined as the time when the 63% of the maximum longitudinal component is recovered (Figure 2). This value is different for many tissues; it is related with the efficiency of transferring the energy from the nuclei to the lattice. This transfer will increase in efficiency whenever the movement of the molecules of the tissue matches to the Larmor frequency.

Solids have a long T_1 because they do not allow the movement of the molecules, so the transfer of energy is more difficult. Pure liquids have also a long T_1 because, even though the allow movement of the molecules, this movement is not close to w_0, since they move too fast. Viscous media like fat have speeds closer to the Larmor frequency and therefore have shorter values of T_1.

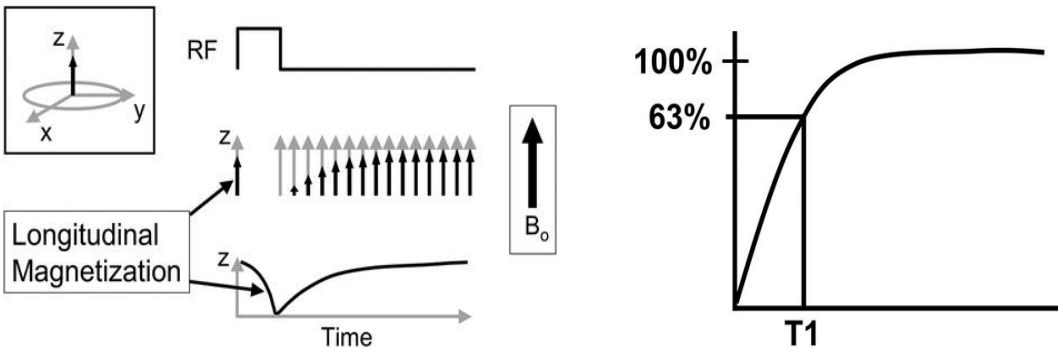


Figure 2 Behavior of longitudinal relaxation [6].

Transversal Relaxation

Transversal relaxation is the loss of the transversal component. It is also called spin-spin relaxation and it is characterized by the T_2 parameter, defined as the time when the 63% of the transversal component is lost (Figure 3). It occurs at a faster rate than T_1. Transversal component is lost because of spin dephasing, which is due to the interaction between spins that create random local variations of the magnetic field in the tissues. However, transversal relaxation occurs at a faster rate than in theory, because the scanner cannot create a totally homogeneous B_0. This shortening of T_2 caused by magnet field inhomogeneities is characterized by T_2* parameter.

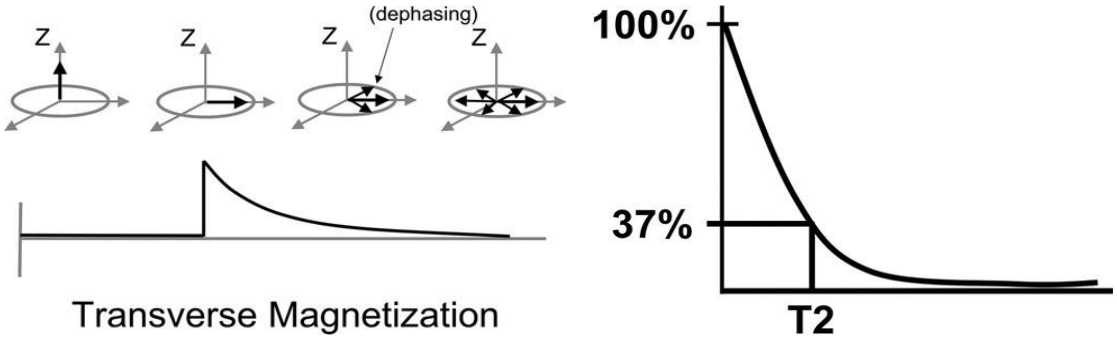


Figure 3 Behavior of transversal relaxation [6].

Basic sequences used in MRI

Spin Echo: These sequences use a trick to recover dephasing produced by field inhomogeneities. After certain time after having applied a 90º RF pulse, they send a 180º RF pulse to revert this dephasing and forming an echo of the signal. Thanks to this pulse, Spin Echo images do not depend on T_2^* but on T_2.

Gradient Echo: Instead of using a 180º pulse, gradient fields are switched on and off to dephase and rephase the signal in order to produce an echo. These sequences have a flip

angle smaller than 90º; therefore, they can be faster and allow shorter repetition times. These sequences cannot revert the T_2^* effect, thus they are usually weighted in T_1 or T_2^*.

As transversal and longitudinal relaxation are independent from each other, the sequences can be adapted by changing some parameters in the acquisition -as repetition time and echo time - in order to weight the images in T_1 or T_2. Repetition time (TR) is the time between excitation pulses and Echo Time (TE) is the time between the excitation pulse and data read-out. TR length determines how much time the sample has to relax in longitudinal direction and TE how much time the sample has to relax in the transverse phase. Images with long TR and TE are T_2 weighted (Figure 4) while images with short TE and short TR are T1 weighted.

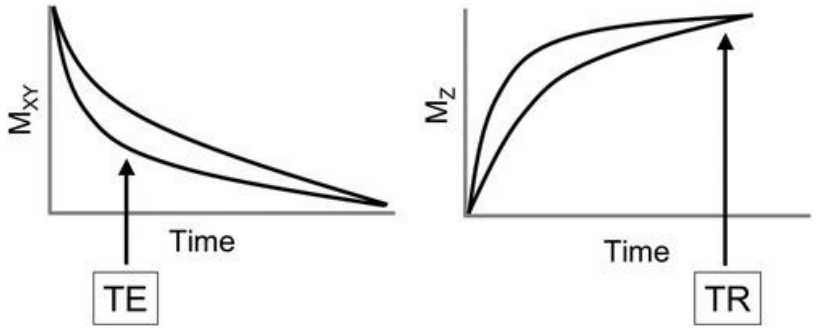


Figure 4 TE and TR parameters required for T_2 weighted images [6].

1.2. Perfusion in MRI

Magnetic Resonance Imaging can study how oxygen and nutrients are delivered to the brain tissue. By assessing perfusion a wide range of diseases can be characterized and diagnosed. Perfusion techniques can be classified into using or not an exogenous contrast agent, which in most cases is a gadolinium based contrast. Methods with injection of contrast agent typically provide higher sensitivity and spatial resolution and are more widely used in routine clinical applications.

1.2.1. Perfusion quantification in MRI

There exist several methods for assessing brain perfusion, which use different approaches to evaluate the microvasculature of the cerebral tissue. The most common methods and their characteristics are summarized in Figure 5.

The methods that involve the injection of a bolus of contrast are Dynamic Susceptibility Contrast (DSC) and Delayed Contrast Enhancement (DCE). DSC consists on tracking the injected bolus of contrast by capturing the change of the MRI signal in T_2/T_2^* weighted images during the first pass of the contrast through the brain. It is the most used technique and software to post-process this sort of perfusion images is widely available. DCE also needs an external contrast, but measures different perfusion parameters that DSC, because it can also assess the integrity of the blood brain barrier

(BBB). It measures changes in signal of T_1- weighted images after injecting a bolus of an external contrast.

Finally, Arterial Spin labeling (ASL) is a method that does not need the use of any external contrast; it utilizes patient's water molecules as tracers. At first, the brain is imaged, then, the magnetization of water molecules of an area proximal to the brain is inverted. After waiting for some seconds, these water molecules arrive to the Field of View –in this case, the brain- and another image is acquired. Finally, both images are subtracted. ASL is completely non-invasive as it does not make use of any external contrast. However, it provides low Signal to Noise Ratio and relatively low temporal resolution and the only perfusion parameter that can be directly computed is the Cerebral Blood Flow [7].

	DSC	DCE	ASL
Full term	Dynamic susceptibility contrast	Dynamic contrast enhanced	Arterial spin labeling
Bolus handling	Bolus tracking	Bolus passage	Bolus tagging
Acquisition point	First pass of contrast agent	Accumulation of contrast agent	Accumulation of tagged blood
Exogenous or endogenous	Exogenous method	Exogenous method	Endogenous method
Contrast media	Intravenous bolus injection of Gd-based contrast agent	Intravenous bolus injection of Gd-based contrast agent	Without contrast agent
Tracer	Non-diffusible blood pool tracer	Flow or permeability-limited diffusible tracer	Diffusible tracer
Relaxation mechanism	T_2/T_2^* relaxation	T_1 relaxation	Magnetic labeled blood T_1 relaxation
Effect	Increased susceptibility effect	T_1 shortening effect	Blood magnetization inversion
Signal behaviors	Decreased signal	Increased signal	Subtracted signal

Figure 5 Different types of perfusion techniques [8].

1.2.2. Dynamic Susceptibility Contrast (DSC)

DSC is the procedure most widely used for brain perfusion. This technique consists on acquiring several images before, during and after the injection of the bolus of the external contrast agent, lasting the total series for less than two minutes. The contrast used is paramagnetic, in most cases a gadolinium-based contrast agent. Gadolinium favors transversal relaxation; therefore, the MRI signal drops when the bolus of contrast reaches the tissue. This change is observed in T_2 or T_2^* weighted images; thus sequences with proper TR and TE are selected. Image acquisition must be fast in order to detect how the MRI signals changes along time; therefore, it requires rapid imaging sequences such as Echo Planar Imaging (EPI). EPI reduces acquisition time and motion artifacts, but it is rather susceptible to field inhomogeneities [9]. DSC acquires several repetitions of images; the intensity profile that follows each voxel of the brain is similar to the curve in Figure 6.

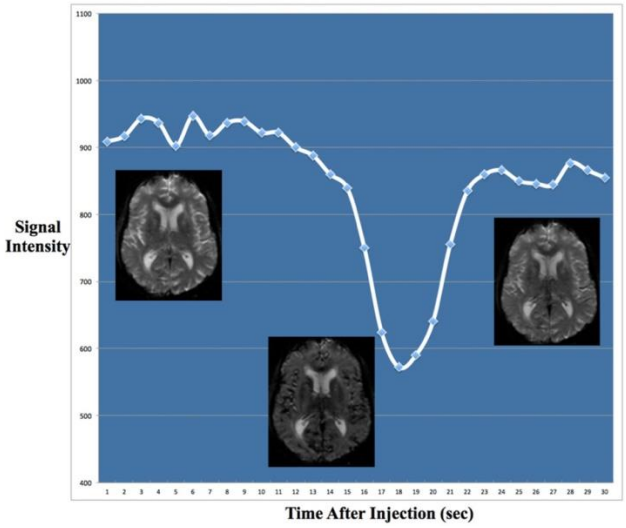


Figure 6 Representation of the signal intensity of the brain during the injection of contrast (DSC) [4].

In order to measure perfusion in a quantitative manner, different perfusion parameters are computed. These parameters behave differently in healthy tissue, areas with ischemia and tumors (Figure 7). One of the parameters measured is the cerebral blood flow (CBF), which is this volume of blood per unit of time that arrives to the tissue. It is usually used to assess tissue viability as it is related to the delivery of oxygen and nutrients. In regions of ischemia, CBF is considerably reduced while in brain tumors the flow typically increases. In ischemic regions, the time at which the contrast arrives to the tissue, which is defined by the parameters 'time to peak' and 'mean transit time', also increases [10].

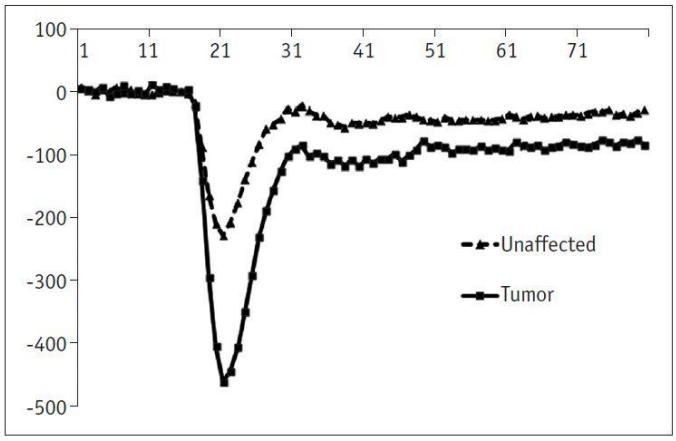


Figure 7 Hemodynamics of gadolinium in DSC [8].

Dynamic Susceptibility Contrast makes some assumptions that are not totally true and can be a source of error. In order to compute the perfusion parameters, DSC assumes no pooling of contrast and no recirculation of the tracer [8]. Gadolinium-based contrast is not retained in the extracellular space of the brain in normal conditions because of the Blood Brain Barrier; however, micro ruptures or a total BBB breakdown due to acute

ischemia might lead to contrast leakage, which violates DSC quantification assumptions. Another incorrect hypothesis is that of no recirculation of the contrast. As gadolinium is not completely absorbed by the kidneys during the first passage, the MRI signal recovery is much slower in reality than in theory. The problem of recirculation can be alleviated by fitting the time curves to a gamma function [11].

1.3. Blood Oxygenated Level Dependent Signal (BOLD)

Blood oxygenated level dependent contrast is a mechanism used in functional imaging to study the activation of different parts of the brain. It is based on the magnetic differences that exist between oxyhemoglobin and deoxyhemoglobin, as they are diamagnetic and paramagnetic, respectively. Dexoyhemoglobin facilitates transversal relaxation creating local field distortions, thus lowering T2-weighted imaging signal. This creates a natural difference between oxygenated and deoxygenated areas of the brain. BOLD can measure the activity of the brain because, when an area of the brain is activated, it needs more oxygen supply [12] and triggers a physiological mechanism (neurovascular-coupling) that increases the CBF in that area. However, as it arrives more oxygen than necessary, activated areas become slightly brighter than the rest of the brain. BOLD is usually studied under conditions of normoxia and the changes of MRI signal are very small.

1.4. State of the Art

Nowadays, perfusion is mainly assessed using invasive methods that make use of gadolinium-based contrasts. Gadolinium involves higher costs and it is contraindicated for patients with kidney diseases. Moreover, it is administered intravenously, with all the associated inconveniences. Alternatives haves been proposed to these methods: although BOLD is primarily used to assess regional brain activation, it is also being used, along with other perfusion techniques, to assess kidney perfusion [13]. Some studies do also measure perfusion making solely use of BOLD signal. Although BOLD is dependent on CBF, it does not give a direct measure of it [14]. Recently, it has been discovered that very small frequency amplitude modulations of the MRI signal appear when performing resting-state BOLD MRI - with the patient in resting state. These fluctuations seem to be proportional to venous oxygenation and Cerebral Blood Flow. This method has been proposed for studying perfusion in preclinical studies, though there are no clinical studies that assess its robustness yet [15].

To our knowledge, there are no previous publications that assess perfusion through conventional techniques such a DSC or DCE using blood oxygenation level as a non-invasive contrast. Some studies have used changes in saturation of oxygen in a prolonged period of time using ASL technique in order to obtain perfusion parameters, since under normal conditions ASL only enables to obtain CBF. However, the change in saturation modifies the physiological conditions of the subject, leading to an underestimation of the CBF [16].

This project explores a novel approach for the assessment of perfusion, following the steps of a previous bachelor thesis by A. Romanillos [17]. Romanillos studied the feasibility of using short changes in the fraction of inspired oxygen for studying brain perfusion using Magnetic Resonance Imaging, observing a decrease in MRI signal when the concentration of inspired oxygen was reduced for a brief period of time. The purpose of this method was to change the relative concentration of oxyhemoglobin in a bolus of blood without altering the physiological conditions of the subject. Although the project found correlation of these two events, several necessary improvements were identified. Among them are the synchronization of the devices used in the experiment and the comparison of the results with regular gadolinium-based contrast post-processing the images to evaluate quantitatively the perfusion parameters.

2. Motivation and Objectives

2.1. Motivation

The study of brain perfusion is involved in the diagnosis of several brain conditions, such as ischemia, tumors and neurodegenerative diseases. Currently, brain perfusion is widely studied with MRI using DSC, which relies on the principles of tracer kinetic modeling to assess the cerebral blood flow. This tracer is an external agent, typically containing gadolinium, which requires intravenous injection, higher costs and involves several adverse effects, being contraindicated for patients with kidney diseases [18] and pregnant women. It might also trigger hypersensitivity reactions. Furthermore, the use of this contras agent involves a certain amount of error. One of the sources of this error is contrast extravasation due to BBB disruption [2]. This leads to a misestimate of perfusion parameters, as one of the fundamental principles of DSC analysis states that there cannot be retention or pooling of contrast. Another source of error is that kidneys do not completely eliminate gadolinium during the first passage which produces recirculation of the contrast; therefore, the curve of MRI signal when the contrast goes through the tissue is not symmetrical, being the return to the baseline much slower than the entrance of the contrast[11].

One of the alternatives used nowadays for assessing perfusion is ASL. This technique does not make use of an external contrast, but it is not as widely used as DSC and DCE, as it has lower SNR and just only one perfusion parameter can be directly calculated (CBF).

For all these reasons, our hypothesis is that using blood itself as an endogenous contrast in DSC may be an excellent non-invasive solution that will avoid the adverse effect of gadolinium. As BOLD principle states deoxyhemoglobin is paramagnetic, consequently, a bolus of deoxyhemoglobin can be used as contrast for DSC, by changing FiO_2 for a small period of time. This approach will circumvent problems caused by gadolinium and will render the technique completely non-invasive. DSC analysis needs an estimation of the arterial input function (AIF) in order to compute accurate perfusion parameters by using blood as endogenous contrast, the AIF can be given by the saturation of oxygen in blood which can be easily measured with a pulse-oximeter.

To our knowledge, there are no previous studies that make use of brief changes in fraction of inspired oxygen as a contrast agent for DSC. Although there is abundant research about perfusion and BOLD effect, studies were mainly focused on regions of the brain, studied under regular respiration in such a way that changes in saturation are very small. There are also studies of changes in FiO_2 for a long period of time, but on ASL technique [16].

In conclusion, this project constitutes an innovative approach for the assessment of perfusion, perhaps, offering a more efficient and safer alternative to the current Dynamic Susceptibility Contrast method and, therefore, providing a remarkable socio-economic impact, which is further describe in Section 3.

2.2. Objectives

The main objective of the project is the study of brain perfusion in an animal model performing a novel approach of Dynamic Susceptibility Contrast, using brief decrease in SpO2 -in other words, a bolus of deoxyhemoglobin- as an endogenous contrast. The specific objectives of the project are:

- Charactering the SpO_2 curve when the FiO_2 is decreased for a brief period of time in a rodent animal model.
- Selecting and optimizing an appropriate MRI sequence for acquiring the images to observe how changes in FiO_2 affect MRI signal.
- To implement the necessary synchronization of hardware and software to control the whole system avoiding possible delays.
- To carry out the experiment in both healthy rats and rats with an induced stroke
- Designing and implementing a MATLAB software for the evaluation of perfusion parameters.
- Comparing endogenous contrast experiments with gadolinium studies

3. Socio-economic impact and regulatory framework

3.1. Socio-economic impact

Social Impact

The possibility of assessing brain perfusion using a non-invasive contrast may benefit patients undergoing this kind of procedure. DSC is the most widely spread brain perfusion technique; therefore, this proposal will directly involve a significant amount of studies. Gadolinium-based contrast has different drawbacks and risks for the patients that may be overcome using changes in FiO_2, which is a completely noninvasive procedure that involves no risks for the patient. The disadvantages of gadolinium are:

- Gadolinium is contraindicated for patients with kidney diseases, since the administration of this contrast has been associated to the development of nephrogenic systemic fibrosis in patients with renal conditions [18].

- Gadolinium based contrast is also contraindicated for pregnant women, since it crosses the placenta and the kidneys of the fetus are not developed yet and gadolinium is likely to accumulate in these organs [18].

- Recent studies suggest that gadolinium may accumulate in the brain –dentate nucleus and globus pallidus – in patients who have gone through repeated administrations of the contrast. This accumulation might be linked to secondary progressive disease subtype multiple sclerosis [27].

Economic impact

This proposal also reduces the cost of the Dynamic Susceptibility Contrast procedure. Gadolinium-base contrast is commercialized as Gadovist 1 mmol/ml by Bayer Hispania S.L. (Madrid, Spain), a bottle of 15 ml costs 169.60 euros and it is intended only for a single use. The use of $Sp0_2$ as an endogenous contrast eliminates the costs of gadolinium-based contrast as well as the additional costs of the injection procedure.

3.2. Regulatory Framework

As the study does not involve humans, the only regulatory and ethics concerns involve the use of small laboratory animals for the experiment. All animal procedures were approved by the Animal Experimentation Ethics Committee of Hospital General Universitario Gregorio Marañón (ES280790000087) and the Ethics Committees of CNIO and the Carlos III Health Institute, Madrid, and performed according to European regulations (2010/63/UE) and National regulations (RD 53/2013).

3.3. Budget estimation

The expenses of this project have three main cost sources: human resources, contracted services from "Instituto de Investigación Sanitaria Gregorio Marañón" and working material.

Human resources:

Service	Time	Cost/hour (€)	Total costs (€) (gross salary)
Engineer	600 hours	15	9000
Supervisor	150 hours	30	4500
		TOTAL	13500

Table 1 Human resources costs.

Contracted services (Contracted services include the material and the service itself. The work is carried out by a qualified imaging technician):

Service	Time	Cost/hour (€)	Total costs (IVA is not included) (€)
Bruker 7/20	6 hours	91	546
Anesthesia	6 hours	10	60
Technician	6 hours	17	102
Injection of gadolinium	2 times	10 (first time) 2 (next times)	12
Stroke surgery	1	33	33
		TOTAL	753 + IVA (21%)

Table 2 Contracted services costs.

Material:

Service	Quantity	Unit cost (€)	Total costs (€)
MATLAB annual license	1	800	800
Laptop	1	850	850
Cables - welding	Several		20
RS-232-USB converter	2	20	40
		TOTAL	1710

Table 3 Material costs.

Source of costs	**Cost** (€)	**IVA (21%)**	**Final cost** (€)
Human resources	13500		13500
Contracted services	753	158,13	911,13
Material	1710		1710
		TOTAL	16121,13

Table 4 Total costs.

4. Materials & Methods

4.1. General design

The overall aim of the project is to assess if changes of FiO_2 can be used as an endogenous contrast for assessing perfusion with DSC technique. To validate our method, we conducted comparative experiments using gadolinium-based contrast agent on a healthy and stroke-induced rat.

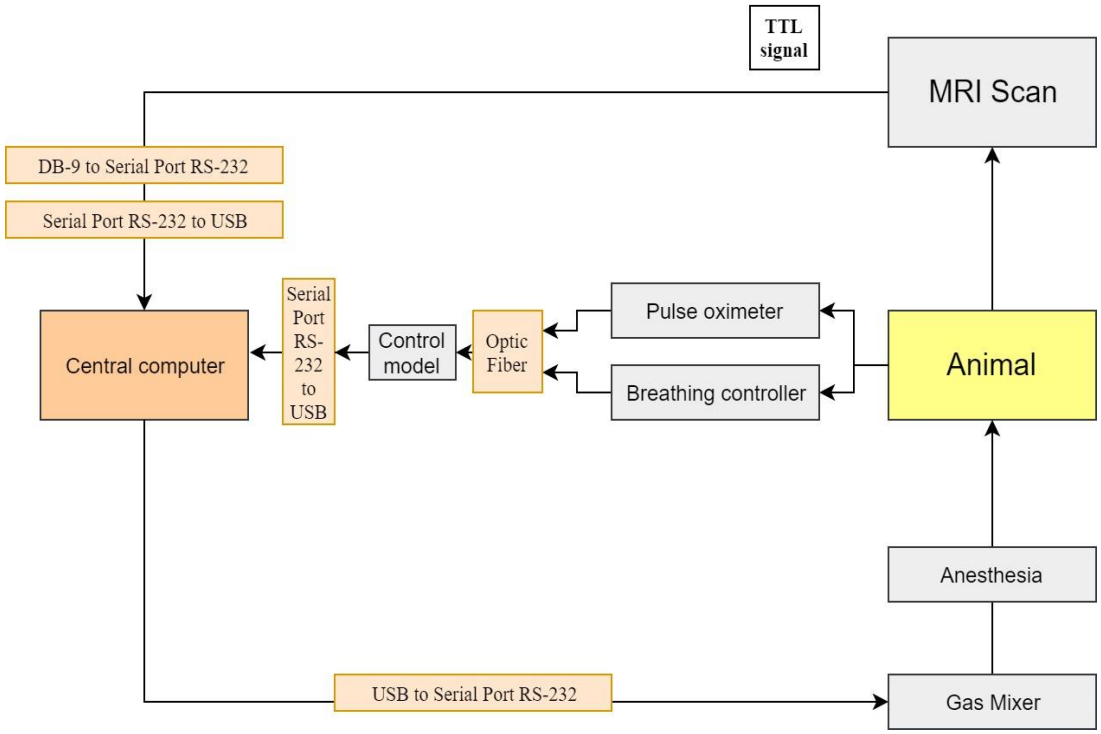


Figure 8 General design of the proposal.

The workflow in Figure 8 represents the general design of the experiment and how the different devices are interconnected. MRI images are acquired during the process to assess cerebral perfusion. In order to acquire these MRI images, the animal is placed inside the gantry of the MRI scan. During image acquisition the fraction of inspired oxygen of the subject, in this specific case a rat, is decreased for a brief instant of time. The animal is carefully monitored by a pulse-oximeter and a breathing controller. Both devices are connected to a control module by optic fiber. This control module is connected to the central computer which processes all data.

The experiment switches from two different FiO_2 concentrations: first stage 100% O_2; second stage, 100% N_2. During the acquisition, the gas mixer and the MRI scan are

automatically synchronized in order to avoid delay if a human operator is involved. At the beginning of the experiment, the animal is administered 100% O_2. When the MRI scan starts acquiring images, it sends a TTL signal to the control computer. The computer processes this information using a MATLAB code and sends a signal to the gas mixer 30 seconds after the beginning of the acquisition. This signal switches the output of the gas mixer to 100% N_2 maintained during 25 seconds. After this time, the original value of 100% O_2 for FiO_2 is recovered.

4.2. Materials

4.2.1. MRI scan

MRI images were acquired using a Tesla BioSPec 70/20 USR (ultra-shielded) scanner (Figure 9) from Bruker Corporation (Bruker BioSpin MRIGmbH, Ettlingen, Ge). This scanner is specific for small animals. Only magneto compatible devices can be introduced inside the security perimeter that surrounds the scanner.

Figure 9 MRI scanner used during the project.

Paravision 6 is the standard software for preclinical MRI scans from Bruker Co. It is used for image acquisition and for optimization of the different parameters of MR sequences.

Two different radiofrequency coils are used to generate the radiofrequency field and to receive the echo emitted by the animal. Both coils are from Bruker Co. The volume coil is placed inside the gantry surrounding the sample. Volume coils can work as emitters

or transmitters; in this case it was used as an emitter. In emission is important to have a homogeneous signal and volume coils provide equal signal to all points of the sample. On the other hand, a brain-surface coil was used for receiving the signal, since the shape fit perfectly the area under study. It also provides higher signal to noise ratio than volume coils.

4.2.2. Monitoring of the animal

During the course of the experiment the animal was carefully monitored to visualize the saturation of oxygen and the breathing rate.

A pulse-oximeter was used to measure the SpO_2 of the animal (Figure 10). The pulse-oximeter has a sensor adapted for rodents which goes clipped to rat's foot to measure the SpO_2 and the pulse of the animal. When the fraction of inspired oxygen is lowered, the concentration of oxygen in blood decreases as well; this produces a decrease in the saturation of oxygen that is register by the pulse-oximeter. This device also gives some important information for the project: the Arterial Input Function (AIF). It corresponds to the curve of SpO_2 when the FiO_2 decreases. AIF is the time-dependent concentration of the tracer and it is used to perform the kinetic modeling and computing perfusion parameters. Furthermore, it is also important to assess that a decrease in the saturation has been properly produced.

Figure 10 The left image is the pulse-oximeter module. The image on the right is the sensor that is clipped to the rat's foot.

The breathing rate of the animal was monitored to control if the animal was properly anesthetized. For this purpose, a respiration pillow adapted for small animals is stuck to the animal to control the respiration.

These experiments were carried out with a pulse-oximeter and a breathing controller especially adapted for small animals and magneto compatible. Both devices are from Small Animals Instruments Inc. One of the advantages of using two devices from the same manufacturer is that they work with the same Software, PC-SAM. It displays all the measurements at real time (Figure 11) and has an option of saving them as a text file.

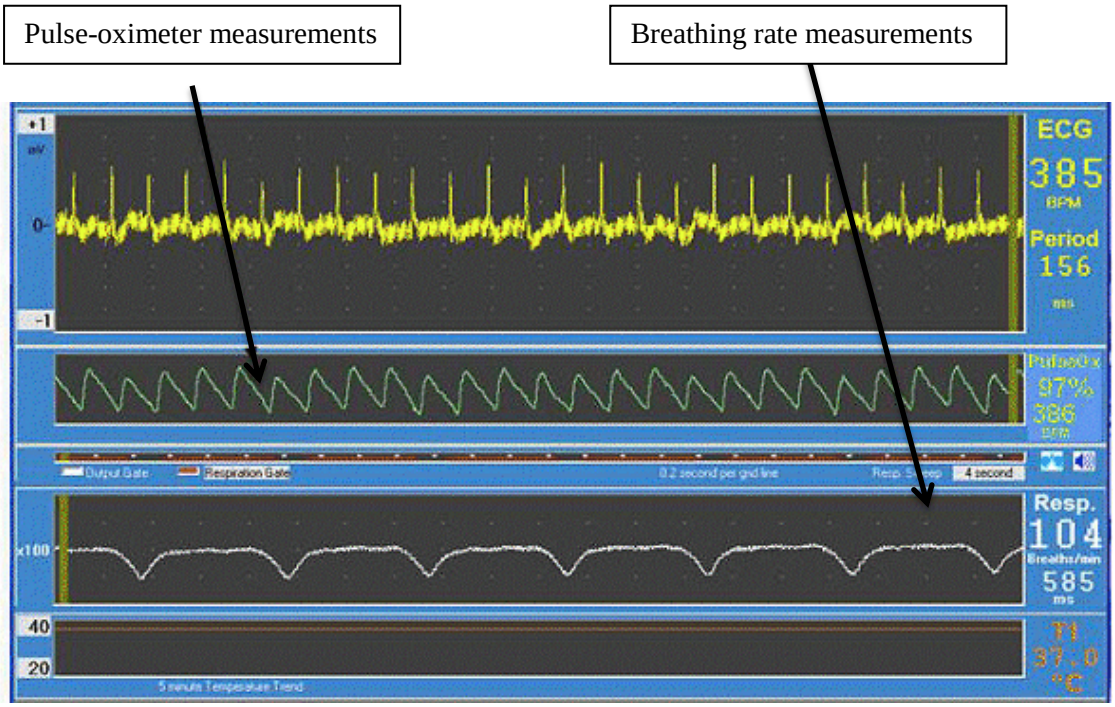


Figure 11 PC-SAM software.

4.2.3. Gas mixer

The fraction of inspired oxygen of the animal was modified with the aid of a gas mixer, GSM-3 model from CWE Incorporate manufacturer (CWE Inc., Pennsylvania) [19]. The gas mixer is not magneto compatible; for this reason, it is located outside the security perimeter of the MRI scanner.

In the front part of the device there are several buttons to control the machine. Up to four different mixtures can be configured, but in our project we are just interested in two mixtures: one of 100% N_2 and another of 100% O_2. GSM-3 allows the user to set the percentages of the mixtures and the total flow. In the left-upper part there is one bottom to select how the device is controlled: locally or remotely. GSM-3 has its own software to control the device remotely, however, one of the objectives of this project was synchronizing the device with the MRI scan and this software does not allowed it. For this reason, a MATLAB code was created to control the device.

Figure 12 Front part of Gas Mixer.

In the back part (Figure 13), there are two inputs for the gases tubes, in our case nitrogen and oxygen, and one output tube for the total mixture. These tubes are connected to the embedded gases at a pressure of 1 bar (Figure 14). There is also one RS-232 connection for the remote mode, which is connected to a cable that goes to the control computer used to change the different mixtures.

Figure 13 Back part of GSM-3.

Figure 14 Gas set up. Left Oxygen, right .Nitrogen

4.2.4. Gadolinium

Gadobutrol (1mmol/ml), market as Gadovist by Bayer AG (Leverkusen, Ge), is a gadolinium-based contrast used to study perfusion in Dynamic Susceptibility Contrast (Figure 15). During this project it was used to compare it to the endogenous contrast for assessing perfusion to determine if similar results can be obtained.

Gadobutrol is administered intravenously dissolved in serum and the concentration of this solution depends on the weight of the animal. 0.5 mmol of Gadobutrol are required for 1000 grams of body weight.

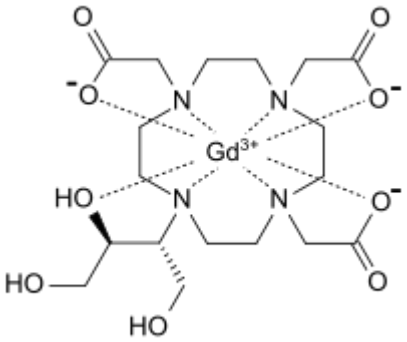


Figure 15 Formula of Gadobutrol

4.2.5. Injection pump

A syringe pump for Harvard Apparatus (Figure 16) was used to administer the external contrast to the rat. The syringe allows to set the desired flow and volume of the sample if the sample and after pressing a button it injects the contrast automatically.

Figure 16 Left image corresponds to the syringe pump used for administering Gadolinium. Right image is the control module of the pump.

4.2.6. Software used for post-processing

After acquisition, the images were examined using ImageJ [20] to observe if there was a change in the signal of the image when the SpO_2 decreases. Once this change was assessed, a MATLAB program and a Graphic User Interface (GUI) [21] were created to post-process the images computing the perfusion parameters and displaying the parametric maps.

4.3. Methods

The project, whose main aim is assessing the feasibility of using changes in FiO_2 as an endogenous contrast, is divided into five different parts:

a) Hardware synchronization
b) Pulse-oximeter characterization
c) Animal studies
d) Image acquisition
e) Image post- processing - GUI Interface

4.3.1. Hardware synchronization

This step consists on synchronizing the MRI scan with the gas mixer. Ideally, the fraction of inspired oxygen should start changing at a fixed time once the MRI scan has started acquiring the images. Before the GSM-3 and scan were synchronized this process was performed manually and had, therefore, some human delay. This automatic synchronization solves this problem and makes the process easier for the user.

The MRI scanner enables to activate a function called *trigger out* that tells the user when the scan starts the acquisition. This function sends continuously a TTL signal of 5 volts, which, at the start of acquisition, changes to 0 Volts. This option is only included in certain sequences, such as EPI. However, it is not included in FLASH sequence in Bruker scans.

In order to communicate the Gas Mixer with the MRI scan a MATLAB script was created (Appendix A).

- MRI scan synchronization:

The scan sends a TTL signal via a coaxial cable. For connecting this cable with the central computer, a db-9 cable was welded to a RS-232 connector and it was in turn connected to a RS-232 to USB converter (Figure 17). Since it is just a binary signal, we were not interested about the data we were reading, just about the change between the 5V to 0V. The outer grid of the coaxial cable was welded to the ground pin of the RS-232 connector and the cable was welded to the Ring Indicator (Appendix B). The Ring Indicator is activated when it detects a low frequency signal – in this case 5V- and deactivated when no data is introduced.

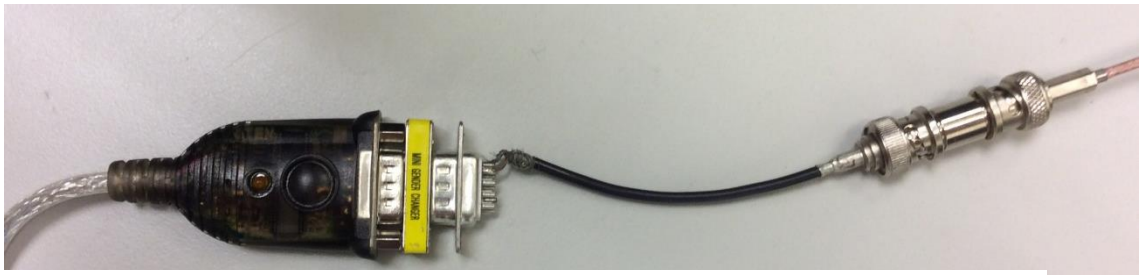


Figure 17 Welded cable connected to one USB-RS232 connector and to the MRI ouput cable.

- Gas Mixer Synchronization:

The Gas Mixer was connected to the computer by a RS-232 to USB converter. GSM-3 has its own software for controlling it remotely; however, this software does not permit the synchronization with the MRI scan, for this reason, a new MATLAB code was created from scratch (Appendix A).

Several mixtures can be configured in the mixer; in this specific case two mixtures were loaded: one mixture of 100% oxygen and another one of 100% Nitrogen. It also gives the possibility to the user of adapting the flow. After performing several experiments, which can be found in section 5.1, 1 l/min was the final flow selected.

- Flowchart of the synchronization:

At the beginning of the experiment the gas mixer sends a 100% O_2 flow which is afterwards mixed with the anesthesia. Once the MRI scan starts acquiring, a TTL signal is sent to the computer. After a time of thirty seconds, the fraction of gas inspired changes from 100% O_2 to 100% N_2. The software maintains this concentration during 25 seconds and then it recovers the initial FiO_2.

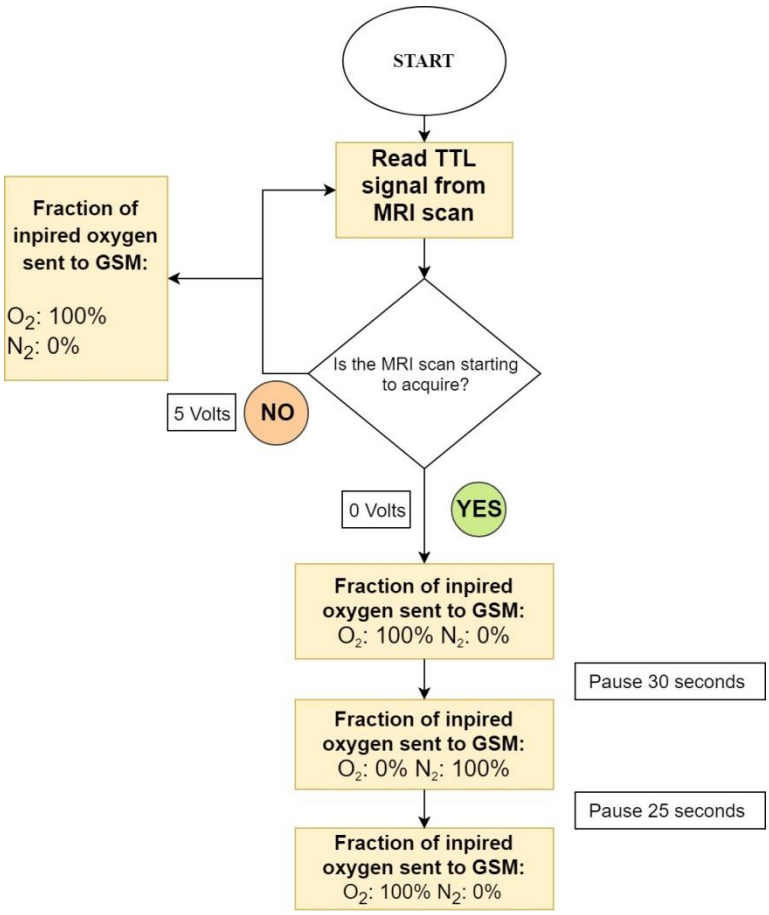


Figure 18 Flowchart of hardware synchronization.

4.3.2. Pulse-oximeter characterization

The shape of the curve of SpO_2 when changing the fraction of inspired gas is influenced by several external factors. Ideally, the shape performed by the bolus of deoxygenated blood should be as similar as possible to an impulse function, and any factor that increases its wide must be minimized. Furthermore, the change of saturation has to be noticeable in order to observe enough differences in the MRI signals. The variables that could potentially determine the curve are carefully examined: different amounts of time of 100% N_2, flow of gases and the length of tubes. The results are described in detail in section 5.1.

4.3.3. Image sequences

In this project two sequences were initially tested: EPI and FLASH. Echo planar images (EPI) are extremely fast gradient echo sequences where the phase encoding step is applied only once; therefore a single image is formed in one repetition time. FLASH (Fast Low Angle Shot) is a gradient echo sequence (GRE) with low flip angle.

In the bachelor thesis of Adrián Romanillos, "Feasibility Study for Brain Perfusion Analysis using Changes of FiO_2 (inspired oxygen percentage) and Magnetic Resonance Imaging"[17], both sequences were tested; however, conclusive results were only obtained for EPI sequences but not for FLASH. The reason why no proper results were obtained is that FLASH images were mostly weighted in T_1 and perfusion changes are only observable using T_2/T_2^*-weighted.

In this project both EPI and FLASH sequences were weighted in T_2^*. However, under T_2^*-weighting conditions FLASH sequences are considerably slower than EPI, which means that the number of images per time acquired is much lower. For a sequence of two minutes length, just 20 samples were obtained for FLASH, while in EPI sequences the rate was 1 image per second (120 images). Therefore, temporal resolution of FLASH is much smaller than EPI for T_2^* images. As the temporal resolution was not good enough to detect the perfusion curve, FLASH sequence was discarded.

Apart of the EPI images acquired during Dynamic Susceptibility Contrast, structural T_1 and T_2 weighted images were also acquired. As contrast and spatial resolution of EPI sequences is low, the parametric maps obtained after calculating the perfusion parameters were overlaid onto the structural images to obtain anatomic information about the animal. The images were exported from the MRI scan to DICOM format.

The parameters selected for EPI were:

Echo Time	60	(ms)
Repetition Time	1000	(ms)
Number of Slices	5	
Image Size	96 x 64	
Field of View	40 x 40	(mm x mm)
Slice Thickness	1	(mm)
Number of repetitions	120	
Duration of the sequence	120	(s)

Table 5 EPI parameters selected for the whole experiment.

4.3.4. Organization of studies

All the studies were carried out with a Winstar rat, one of the most popular breeds used in preclinical research. All of the studies were carried out with a rat of 270 grams of weight. The regulatory framework is further described in section 3. In the first instance, the animal was anaesthetized inside a methacrylate box; using a gas mixture of 79% Nitrogen and 21% Oxygen with sevoflurane (7%) and a gas flow of 1 liter per minute. Once the animal was fully anaesthetized, it was moved to the scan bed. Then, the animal was connected to the mouthpiece of the MRI scan. The anesthesia was maintained during the course of the experiment. It was subsequently decided to keep a 100% O_2, in order to keep the SpO_2 close to 100%. The percentage of anesthesia was adjusted to keep the breathing rate around 30 – 50 bpm (1.5% - 3 %).

Figure 19 Image on the left: Anesthesia used during the experiments. Image on the right: Animal placed inside the scan gantry.

Before stroke-induction surgery two DSC perfusion sequences were acquired: one with endogenous contrast and another one with gadolinium-based contrast. Apart from the EPI sequences, one structural T_1 and one structural T_2 – weighted images were acquired. The same procedure was followed after the stroke induction.

- **Induction of stroke in rats:**

The procedure was carried out by the technicians of the Laboratory of Medical Imaging at Gregorio Marañón Hospital. The surgical model consists on the introduction of a filament inside the external carotid (Figure 20), which is pushed forward the internal carotid occluding the medial cerebral artery. This occlusion triggers the cessation of blood flow causing an infarction in the area. The size of the ictus is determined by the size of the filament and the occlusion time [22]. In this specific case the occlusion time was 30 min. Images were acquired right after the surgery.

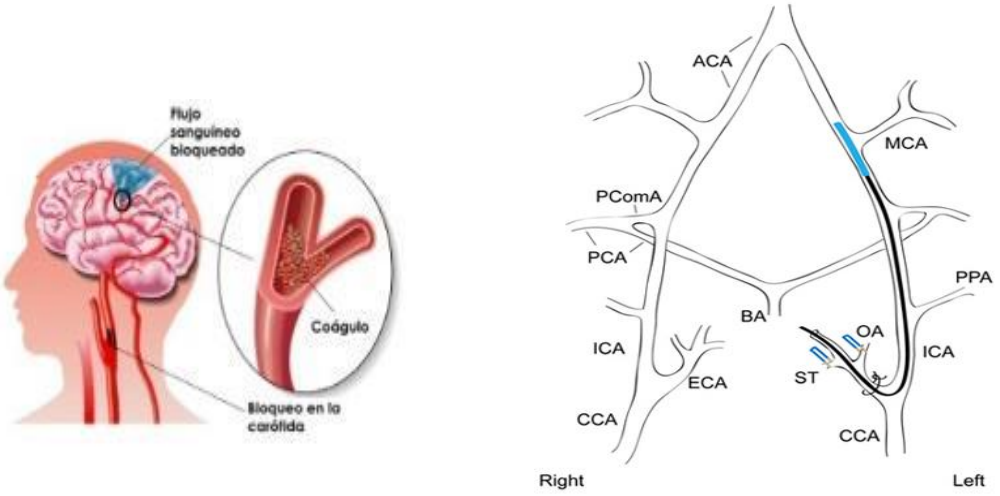


Figure 20 Explanation of the stroke-induction surgery [8].

4.3.5. Parametric maps

After the acquisition of images, they were post-processed to obtain the parametric maps. For this purpose, a MATLAB code and a GUI Interface were created. Parametric maps are a representation of brain perfusion parameters on a voxel-by-voxel basis. The perfusion parameters are used to quantitatively assess brain perfusion; they are useful to diagnose different brain conditions, such as ischemia and brain tumors. The parameters that were computed with the aid of MATLAB were (Figure 21):

Cerebral Blood Volume: It measures the total volume of blood that arrives to a region of brain and it corresponds to the area under the concentration curve. It is measured in ml/100g.

Cerebral Blood Flow: It is the total volume of blood that goes through a region of the brain per unit of time [ml/min/100g].

Mean Transit Time: The mean time that blood employs to go from the artery entrance to the venous way out [seconds].

Time to peak: The time since the injection of the contrast to the maximum loss of signal [seconds].

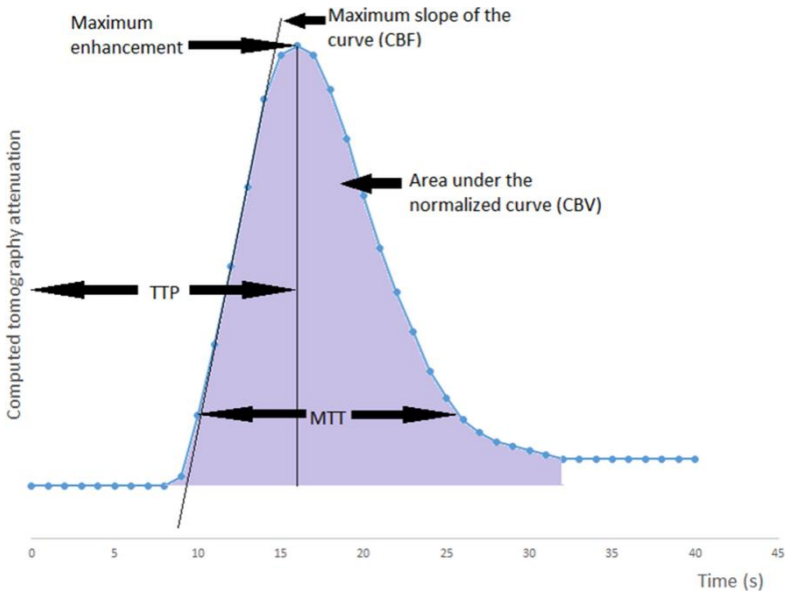


Figure 21 Concentration curve of DSC for the study of brain perfusion [23].

Arterial Input Function (AIF): AIF describes the contrast agent input of the tissue of interest. Ideally, the bolus of the contrast would be modeled as an impulse function; however, the shape of the contrast is more similar to a gamma function. As the AIF changes for every patient, we are now taking data that cannot be comparable among subjects. For this reason, it is required to eliminate the effects of the Arterial Input Function [24].

For gadolinium experiments, the function is carefully extracted from the image, selecting the curve more similar to an impulse function with a greater change in amplitude than the average curves. This process takes some time. However, in our experiment the Arterial Input Function is the curve obtained by the pulse-oximeter; therefore, we just need to take that curve and use it as our Arterial Input Function.

4.3.5.1. Steps for computing the perfusion parameters

The general scheme of image post-processing is shown in Figure 22. The parametric maps (CBV, CBF, MTT and TTP) are computed from two input files: the PC-SAM file where the pulse-oximeter curve, which corresponds to the AIF function, is written and the folder where the images are located.

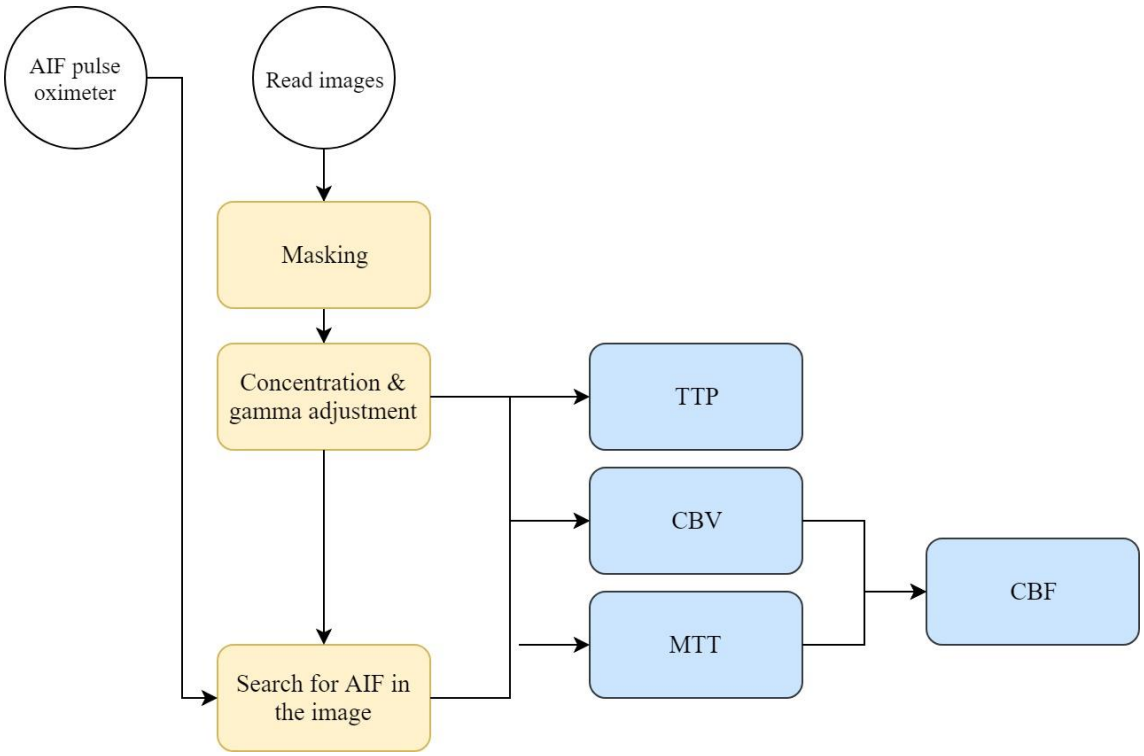


Figure 22 Flowchart of the post-processing MATLAB code.

1. Image loading

The first step consisted on reading the DICOM images, storing them in a matrix and displaying some information about them: Echo Time of the sequences, number of slices and number of time frames.

2. Background noise

In order to get rid of background noise, a mask of the brain was implemented. Noise pixels have low intensity and they must be eliminated to avoid delay in computation. They were eliminated with a simple threshold, which removes pixels of less intensity than the 20% of the median intensity.

3. Calculation of parametric maps

The MRI images are given in units of signal intensity. In order to compute perfusion parameters, these measurements are converted into concentration of the contrast. In our specific case, instead of having as a contrast gadolinium, what is going to be measured is the concentration of deoxyhemoglobin. Concentration is computed for every voxel of the image.

The concentration is related with the MRI signal by this equation [25]:

$$Cm(t) = -k.ln\left(\frac{S(t)}{S0}\right) \qquad \text{(Equation 1)}$$

C_m is the concentration of the contrast, in this case deoxyhemoglobin, in each voxel. K is a constant inversely proportional to the Echo Time. For simplicity, in this project k is $\frac{1}{TE}$. $S(t)$ is the MRI signal of that arbitrary voxel at a time t (Figure 23) and S_0 is the MRI signal of the voxel before the arrival of the contrast. So is the average of the first 5 seconds of image acquisition.

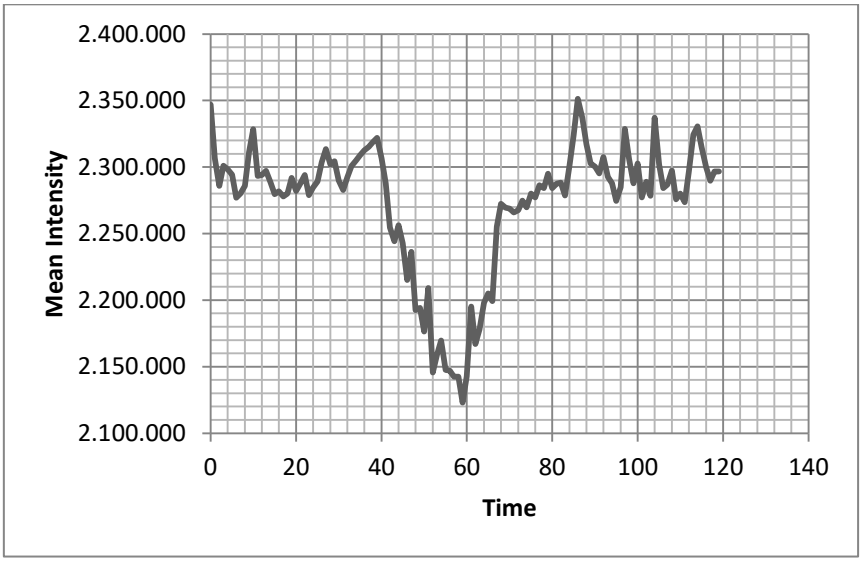


Figure 23 Evolution of the MRI intensity of a voxel of the image along time. It corresponds to S(t).

4. Gamma fitting

In order to eliminate noise oscillations along the time component the concentration curves are adjusted to a gamma equation (Figure 24), the concentration is adjusted to the following equation:

$$Cm(t) = l(t - to)^{\alpha}\, e^{-\frac{t-t0}{\beta}} \qquad \forall\, t > to \quad \text{(Equation 2)}$$

l, alpha and beta are the constants of the gamma function and need to be obtained. t_0 is the time at which the concentration starts to rise and it also needs to be computed. All the parameters are obtained using an algorithm based on based on the Bachelor Thesis of Pedro Macías, "Implementación de una herramienta para medidas de perfusión cerebra en imagen de resonancia magnética bajo ImageJ" [26].

The first step is computing t_0 using the following algorithm [26] :

i. Locate maximum point of the concentration curve (t_{max})
ii. Set $t0 = tmax - 1$.
iii. If $1 - \frac{Cm(t0)}{Cm(tmax)} < 0.5$ & $\frac{Cm(t0)}{Cm(t0-1)} - 1 < 4$ & $Cm(t0-1) > 0$, then $t0 = t0 - 1$.
iv. If the value meets the previous conditions, the search continues. If not, t_0 is set.[26]

For adjusting the voxels values to a gamma function, it is necessary to linearize the equation by taking logarithms at both sides:

$$ln\big(Cm(t)\big) = ln(\mathrm{l}) + \alpha l.n(\mathrm{t} - \mathrm{t}_0) - \frac{1}{\beta}\,(\mathrm{t} - \mathrm{t}_0) \quad \forall\, \mathrm{t} > \mathrm{t}_0 \quad \text{(Equation 3)}$$

This equation is similar to:

$$\mathrm{y1} = \ln(\mathrm{l}) + \alpha.\mathrm{x1} - \frac{1}{\beta}.\mathrm{x2} \qquad \text{(Equation 4)}$$

where

$$y1 = \ln(Cm(t))$$

$$\mathrm{x0} = 1$$

$$\mathrm{x1} = \ln(\mathrm{t} - \mathrm{t0})$$

$$\mathrm{x2} = \mathrm{t} - \mathrm{t0}$$

This is a matrix problem: $\mathrm{Y} = \mathrm{X}\,\mathrm{b}$ (Equation 5), here b is the vector: [ln(l), α, – 1/β]. This problem is solved using singe value decomposition function in MATLAB.

$$b = X^{-1}Y \qquad \text{(Equation 6)}$$

$$X^{-1} = VSU^{-T} \qquad \text{(Equation 7)}$$

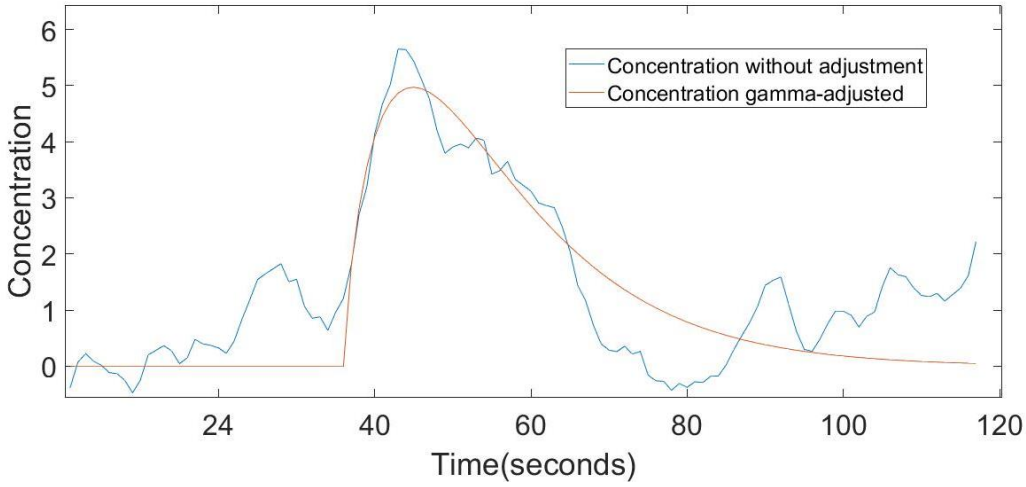


Figure 24 Plot of the evolution of the concentration along time of a random voxel with and without gamma fitting. Gamma fitting reduces noise.

5. AIF adjustment

The shape of the Arterial Input Function is determined by the pulse-oximeter output curve; however, the exact concentration values are not known. For scaling AIF to the concentration values of the images, the curve is compared with the different curves of the different voxels of the images, looking for a curve with a similar shape. The full width half maximum parameter is used to compare the different curves. By definition, AIF curves have higher concentration changes than normal voxels; therefore, curves with higher concentrations than the average were selected. The program selects the five best curves and the user can select the best one among them. Finally, the pulse-oximeter curve is scale to those values.

6. Perfusion parameters [25]

Time to peak (TTP): It is computed at the time at which every voxel reaches the maximum value of concentration.

Cerebral Blood Volume (CBV): The equation used to compute the CBV is:

$$CBV = \frac{k_h}{\rho} \cdot \frac{\int C_m(t)dt}{\int AIF(t)dt} \quad \text{(Equation 8)}$$

K_h is 0.8 and ρ is the density of the cerebral tissue (1.04 g/ml).

Mean Transit Time (MTT): The mean transit time is computed with the following equation.

$$MTT = \frac{\int C(t)dt}{C_{max}} \quad \text{(Equation 9)}$$

C is the concentration without taking into account the particularities of AIF. It is computed performing the deconvolution of C_m with the Arterial Input Function; this procedure is very sensitive to noise:

$$C(t) = C_m(t) *^{-1} \text{AIF (t)} \quad \text{(Equation 10)}$$

It can be approximated with the following matrix equations:

$$\begin{bmatrix} C_m(t_0) \\ C_m(t_1) \\ \cdots \\ C_m(t_i) \end{bmatrix} = \begin{bmatrix} AIF(t_0) & 0 & \cdots & 0 \\ AIF(t_0) & AIF(t_0) & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots \\ AIF(t_i) & AIF(t_{i-1}) & \cdots & AIF(t_0) \end{bmatrix} \cdot \begin{bmatrix} C(t_0) \\ C(t_1) \\ \cdots \\ C(t_i) \end{bmatrix}$$

(Equation 11)

This problem is solved creating the previous matrix of AIF values and applying its single value decomposition to obtain its pseudo inverse- matrix and calculate C(t).

Finally, the Cerebral Blood Flow is calculated using the Central Volume Theorem, which states that the Cerebral Blood Flow equals the Cerebral Blood Volume divided by the Mean Transit Time and is expressed as follows:

$$CBF = \frac{CBV}{MTT} \quad \text{(Equation 12)}$$

4.3.5.2. Graphic User Interface (GUI)

A MATLAB interface was created to develop a visual way of interacting with the user. This application is a rapid and easy way of computing perfusion parameters of DSC technique using changes in FiO_2.

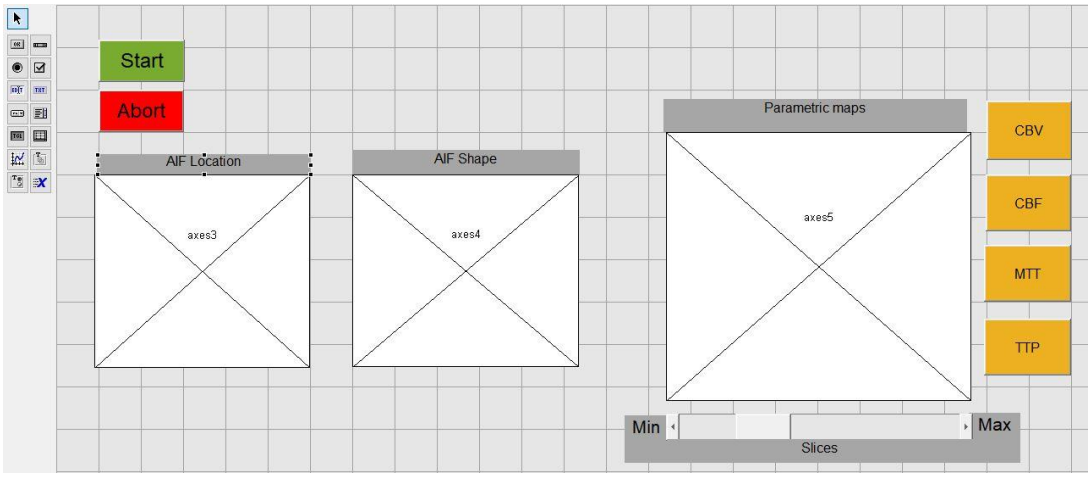


Figure 25 MATLAB interface of the perfusion code.

The final working window contains the following elements:

- Start and abort buttons:
  Each time the user presses the *start* button the program is initialized. There is also another red button to abort the program any time the user desires it.
- Three graph windows where different plots and images are plotted during the execution of the program.

Structure of GUI Interface

1. There is a pop-up window which asks the user for the path of the images and the path of the SA Instruments txt file generated by the pulse-oximeter.

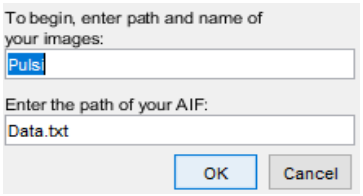


Figure 26 Emerging window of input data.

1. The code reads the images, providing the user information about them.

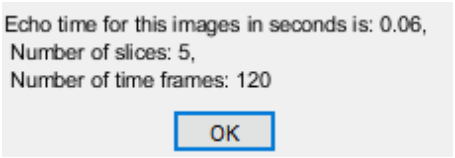
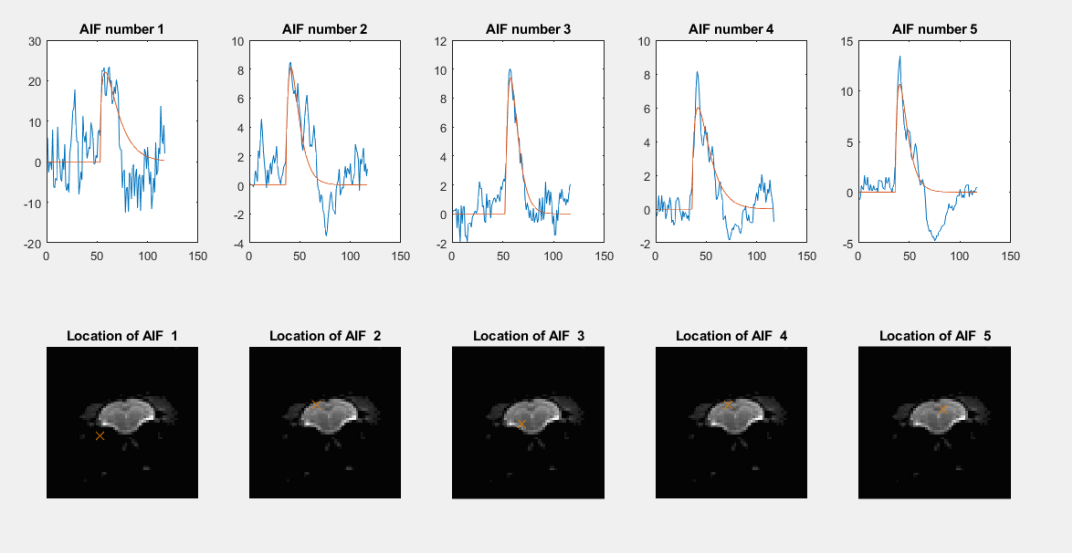


Figure 27 Information provided about the images by the application

2. Image data are converted into concentration and fitted to a gamma function.
3. The program compares the AIF from the pulse-oximeter with the image, selecting the best matches and giving the user the option of choosing the desired one.



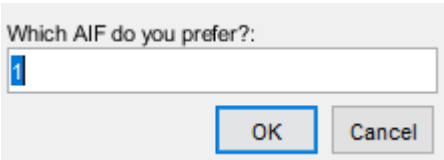


Figure 28 Plot of the different options that the program provides for being the AIF. It also provides their location in the images. The blue curves correspond to the time curves of the image while the red curves are the AIF from the pulse-oximeter.

4. The selected Arterial Input Function is plotted and its location is shown. In the right side of the interface, the user has the possibility of selecting among the different parametric maps computed by pushing one of the four bottoms. Using the sliding bar the user can also move along the different slices of the image.

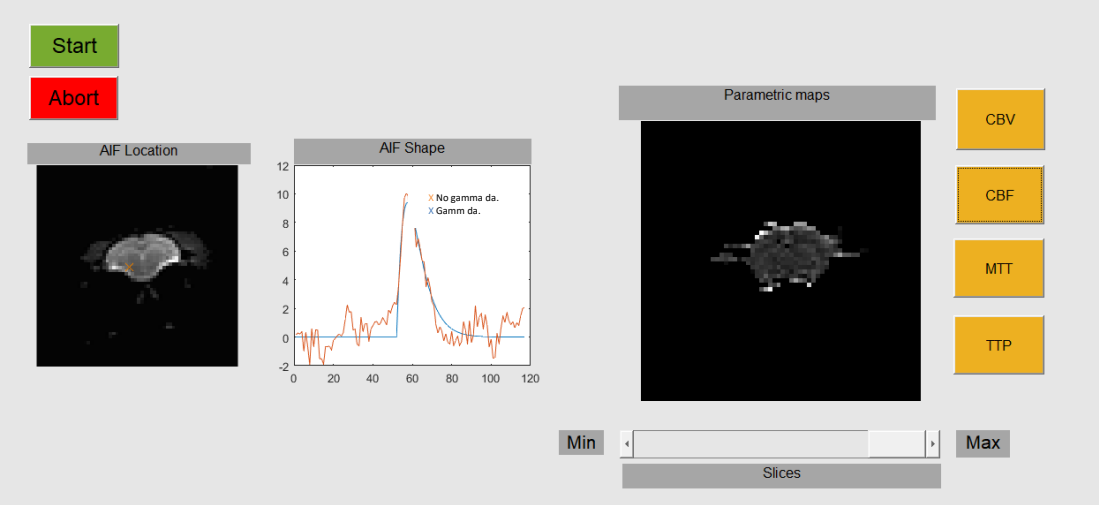


Figure 29 Interface output. In the left side the AIF function and its location are shown.. In the right side of the image the different parameter maps can be displayed.

5. Results

5.1. Pulse-oximeter curve characterization

A previous step to image acquisition consisted in carefully characterizing the shape of the pulse-oximeter curve when a bolus of deoxygenated blood goes through the circulatory system. One important issue is that the change in saturation has to be large enough so as to be easily detected in the presence of noise in further post-processing. For studying the pulse-oximeter behavior a gas mixture of 100% N_2 was sent at the beginning and the mixture changed to 100% O_2 after some time. The plots can be found in Appendix C. The different variables studied were:

- Duration of 100% N_2 stage:

Different durations of N_2 were studied: 20 seconds, 25 seconds and 30 seconds. They were studied using the standard tube and a flow of 600 ml/ min. The parameters shown in Table 6 are the minimum saturation, the time at which the minimum saturation is reached (t_{min}) and the total width of the saturation change for the different durations of nitrogen.

	20 seconds	**25 seconds**	**30 seconds**
Minimum value of $Sp0_2$	72 %	61 %	57 %
t_{min}	42 seconds	51 seconds	52 seconds
Total width of change	18 seconds	31 seconds	38 seconds

Table 6 Quantitative results using different periods of N_2.

- Gas mixture flow:

The gas mixer, GSM-3, allows the user to adjust the flow of the gas mixture. Different flows were examined to determine the most suitable one for the experiment (Table 7). The duration of the nitrogen 100% stage was 25 seconds.

	500 ml/min	**600 ml/min**	**1000 ml/s**
Minimum value of $Sp0_2$	73 %	61 %	37 %
t_{min}	53 seconds	51 seconds	25 seconds
Total width of change	30 seconds	31 seconds	32 seconds

Table 7 Quantitative results using different flow of gas.

- Length of gas tubes:

The length of the gas tubes must be long enough to reach the MRI scanner field of view. As the gas-mixer is not magneto compatible, it has to be kept out of the 5 Gauss area.

To assess the role of tube length, the pulse-oximeter measurements were also taken with a shorter tube of 38 cm and it was compared with the usual tube, which is 789 cm long. If the shape of the curve had changed considerably, another method different to the gas mixer would have been tried to change the FiO_2 (Table 8). The experiments were performed at a flow of 600 ml/min and the Nitrogen duration was 25 seconds.

	Short tube (38 cm)	Usual tube (789 cm)
Minimum value of $Sp0_2$	65 %	65 %
t_{min}	44 seconds	51 seconds
Total width of change	26 seconds	28 seconds

Table 8 Quantitative results using different tube lengths.

5.2. Analysis of MRI signal change

After acquiring the images, they were analyzed to assess if the change in FiO_2 affected the MRI signal. For this purpose, the time curve of whole brain was obtained for both exogenous and endogenous contrast experiments in pre stroke surgery animals. Furthermore, the time curve of pre and post stroke surgery was also plotted to compare the differences.

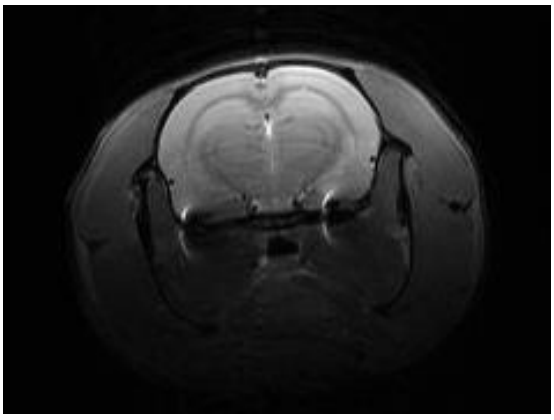


Figure 30 Cerebral axial cut of the rodent (T_2 image).

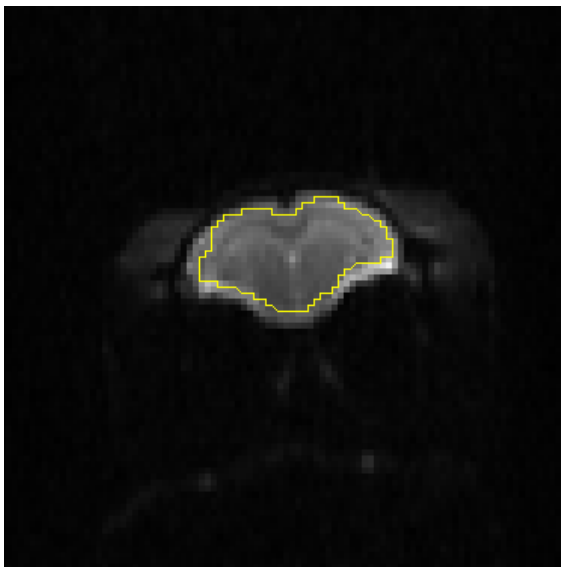


Figure 31 ROI selected to compute the time curve (Echo Planar Imaging).

Plots for experiments N_2-DSC and Gd-DSC pre stroke-surgery are represented in Figure 32 and Figure 33, respectively. In all the experiments the MR sequence was an Echo Planar Imaging and the contrast agent was administered 30 seconds after the beginning of data acquisition.

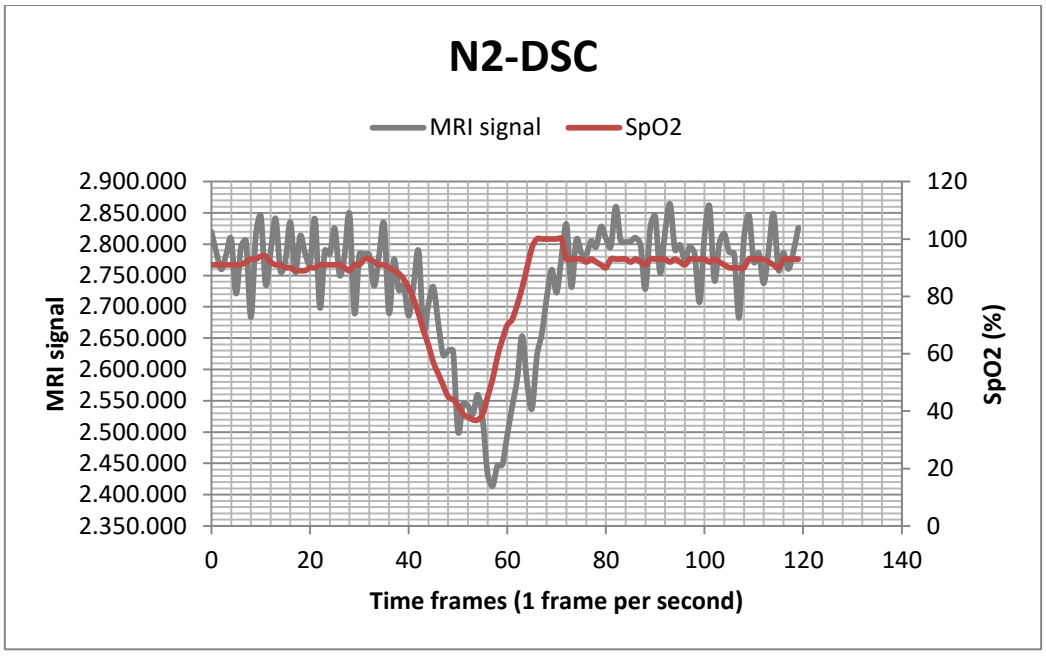


Figure 32 MRI signal and the pulse-oximeter curves using changes in FiO_2 in a healthy rodent.

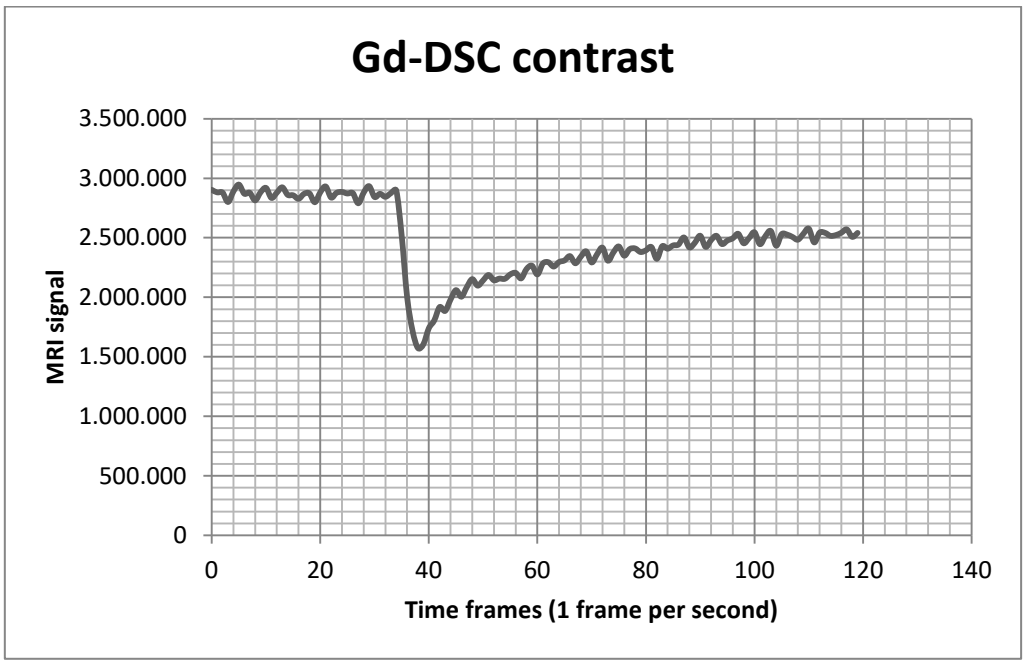


Figure 33 Profile of DSC using Gd as contrast agent in a healthy rodent.

5.3. Structural images of pre and post stroke-induction surgery

The same DSC procedure was carried out before and after stroke-induction surgery. A structural T_2-weighted image of the brain was acquired before and after the surgery to observe the location of the tumor. As it can be in observed in Figure 34 the stroke was induced in the left part of the brain, as it is more evident in slices 4 and 5.

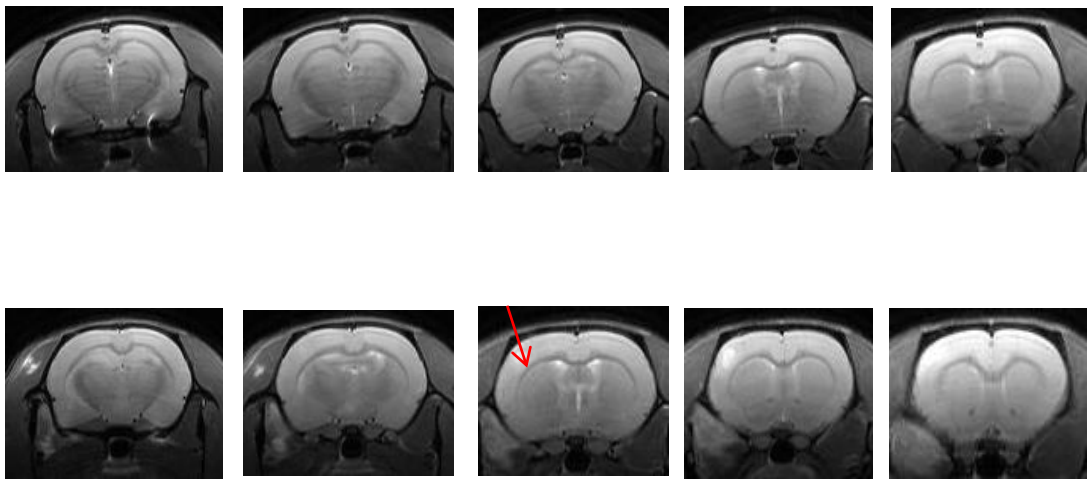


Figure 34 First row: T_2-weigthed images of pre stroke induction surgery of 1-5 brain slices. Second row: T_2-weigthed images of post stroke induction surgery of 1-5 brain slices.

5.4. Analysis of MRI signal before and after stroke-induction surgery

After stroke-induction surgery, the stroke was located in the left brain hemisphere (Figure 35). The time curve of the EPI sequence of the left brain hemisphere was compared with the right one to observe how differently they behaved with both endogenous and gadolinium-based contrasts (Figure 36 and Figure 37).

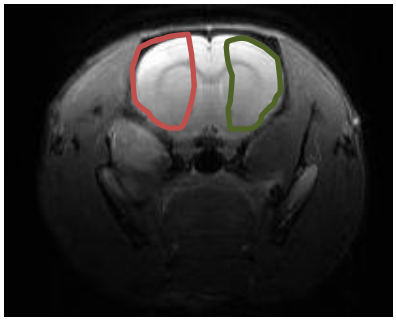


Figure 35 Structural image of the brain. Red area corresponds to the ischemic region and the green area is the non-affected region.

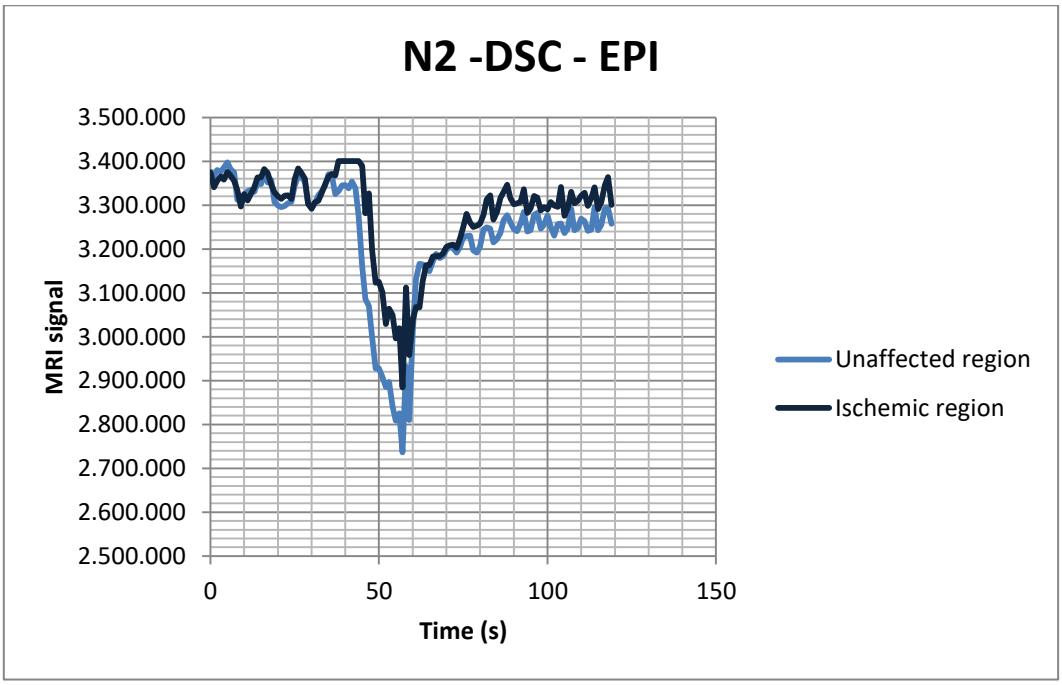


Figure 36 Time curve of DSC using an endogenous contrast of a post stroke-induction surgery brain.

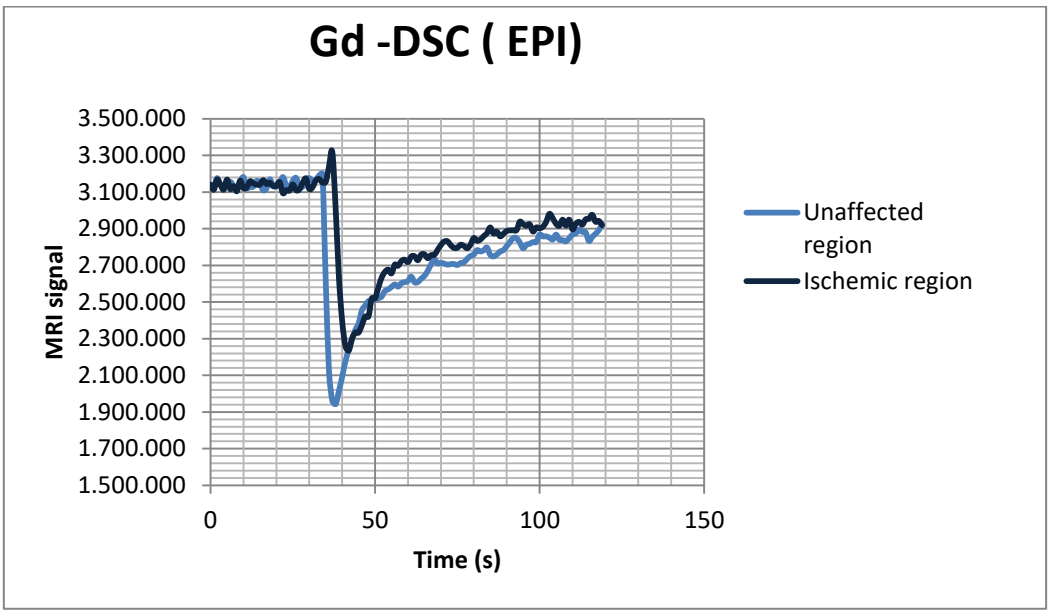


Figure 37 Time curve of DSC using a gadolinium-based contrast of a post stroke-induction surgery brain.

5.5. Parametric maps

The software developed MATLAB allows the user obtaining four different parametric maps: Cerebral Blood Volume (CBV), Cerebral Blood Flow (CBF), Mean Transit Time (MTT) and Time to Peak (TTP). These maps are displayed in the GUI interface together with the Arterial Input Function (AIF) selected by the user and its location in the brain (Figure 38).

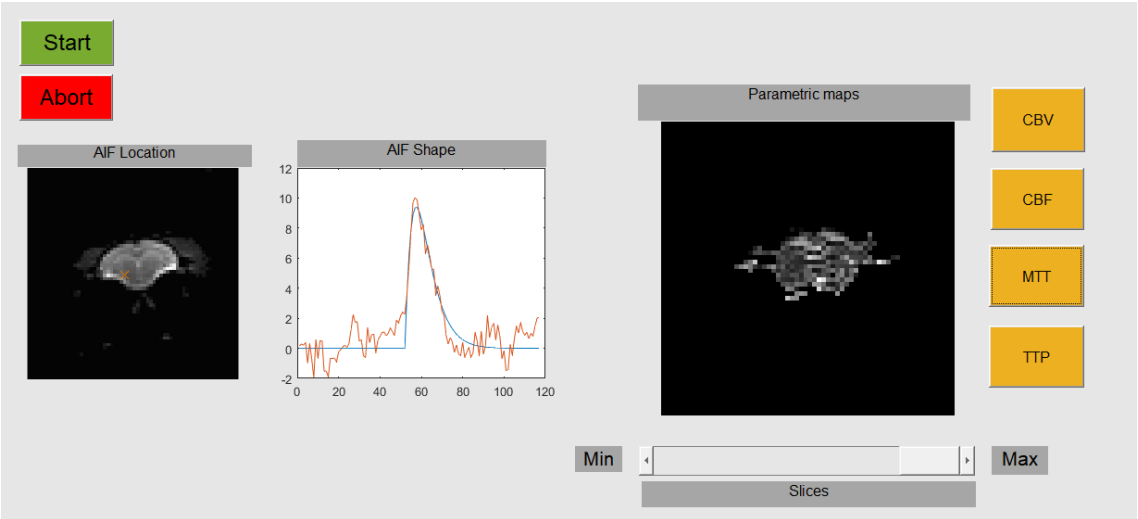


Figure 38 Graphical user interface. In the left part there is the representation of the AIF location and shape. In red we can observe the AIF without Gamma adjustment and in blue the AIF with gamma adjustment. In the right part, the different parametric maps can be displayed.

The parametric maps of the rodent before and after stroke induction surgery using an endogenous were calculated to observe the difference in blood perfusion; they are

represented in Figure 39 to Figure 42 . The parametric maps were overlaid with a T_2-weighted structural image with the aid of ImageJ. For this, the parametric maps were rescaled from a matrix size of 96*64 to a matrix size similar to the structural image (256*256), for rescaling bilinear interpolation is utilized.

Cerebral Blood Volume

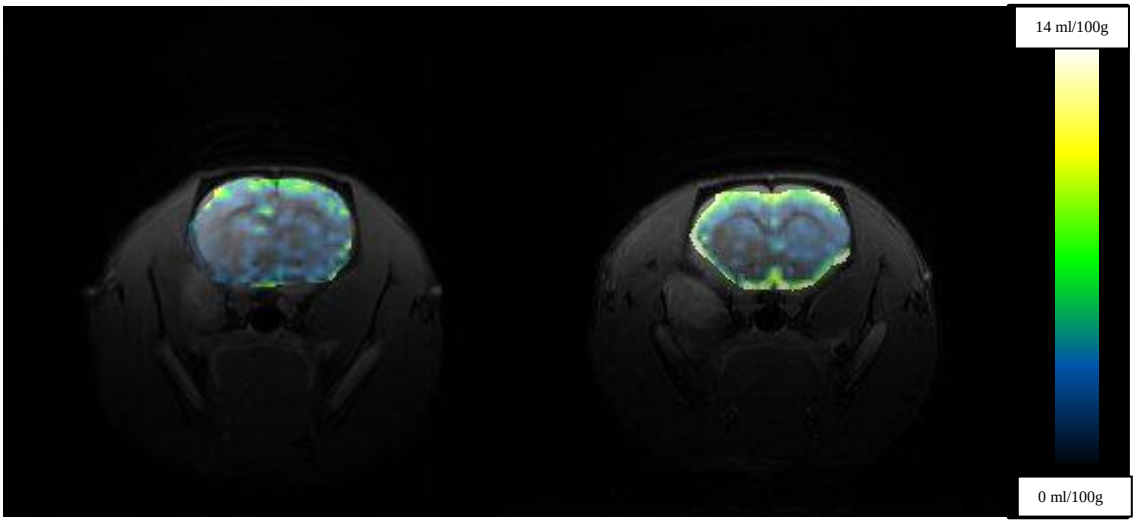


Figure 39 CBV Basal (left) and CBV Ictus (Right) (slice 5).

Cerebral Blood Flow

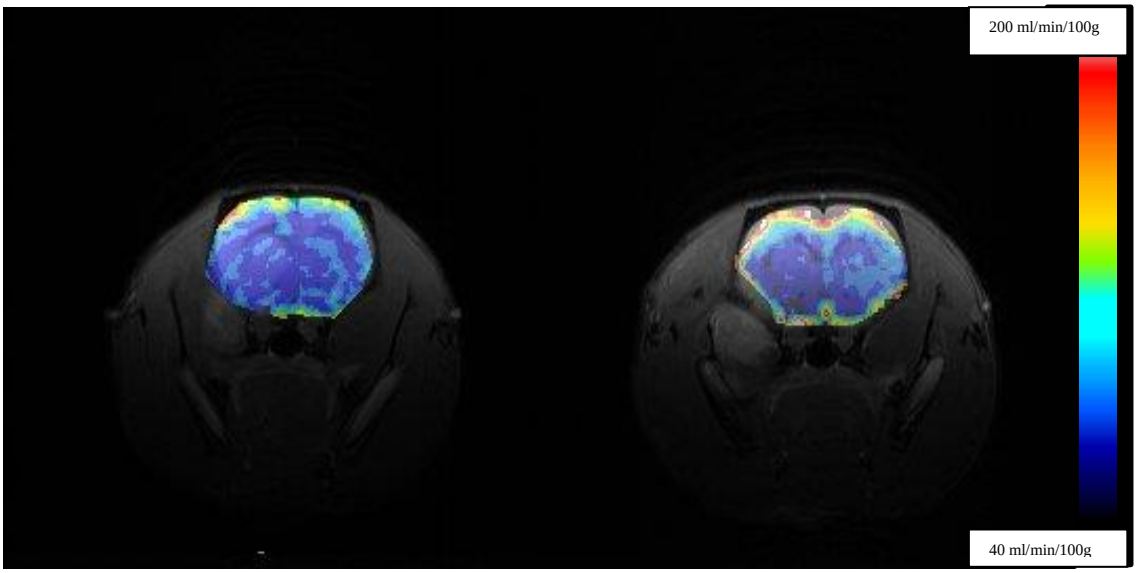


Figure 40 CBF basal (left) and CBF Ictus (right) (slice 5).

Mean Transit Time

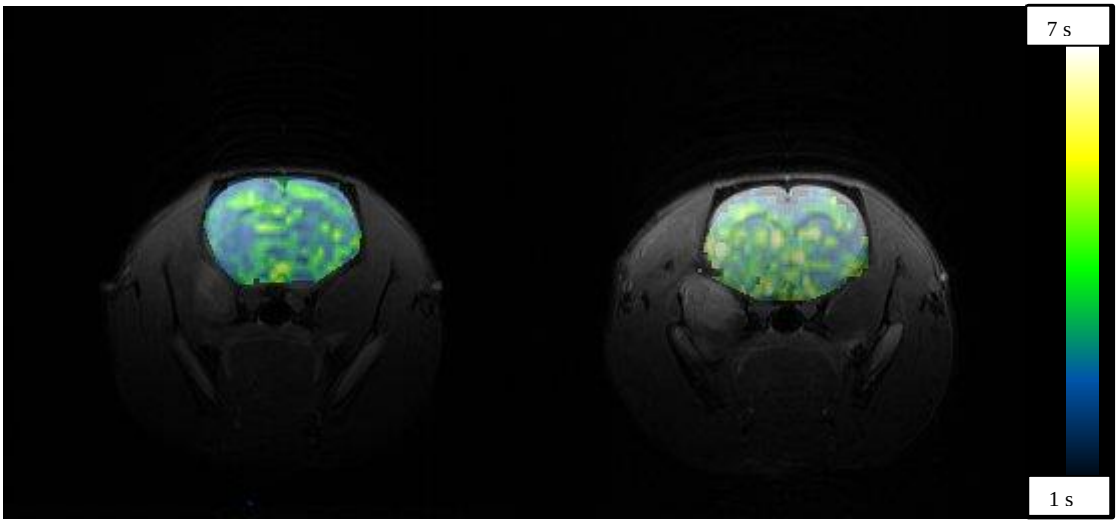


Figure 41 MTT basal (left) and MTT Ictus (right) (slice 5).

Time To Peak

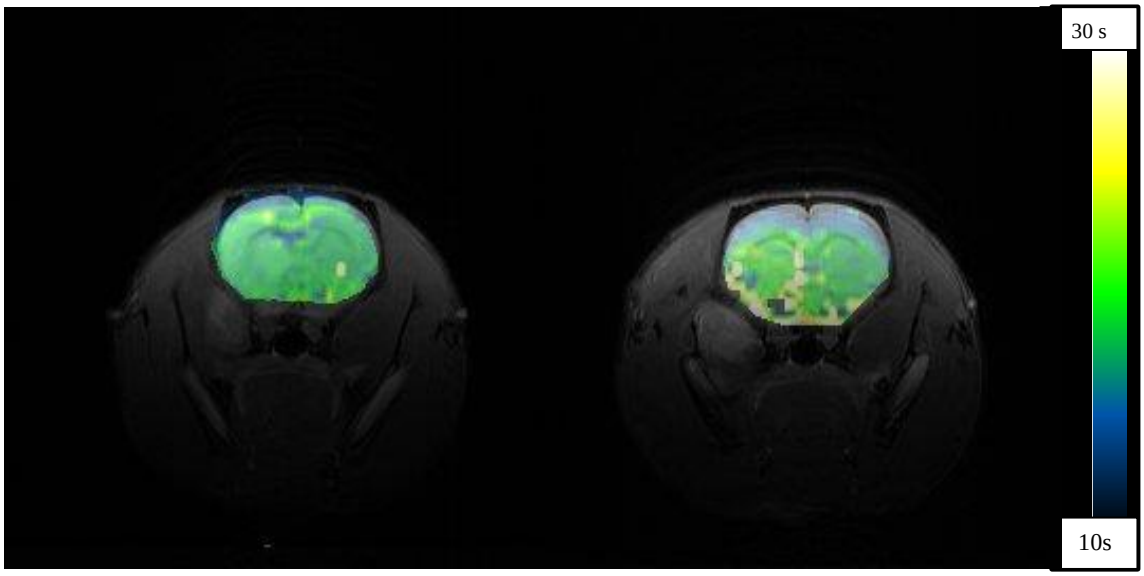


Figure 42 TTP basal (left) and TTP ictus (right) (slice 5).

5.6. Validation of the technique comparing changes in FiO_2 with Gd-contrast images

The technique was validated comparing the images with the exogenous contrast produced by gadolinium administration. The Gd-DSC experiments were carried out right after N_2-DSC; therefore, the animal did not change its position during acquisition and slice geometry was maintained constant. Parametric maps were quantified with ImageJ, comparing the ratio between the ischemic region (left side) and the ipsilateral unaffected region (right side), as it can be seen in Figure 43. The same region was selected in both Gd and N_2-maps in the different slices. Both results (Gd and N_2) were compared in each slice, calculating the coefficient of determination (R^2) calculated by means of linear fitting.

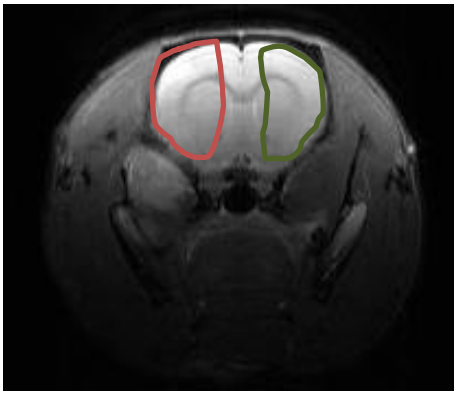


Figure 43 Structural image of the brain. Red area corresponds to the ischemic region and the green area is the non-affected region.

Cerebral Blood Volume:

	N_2 – DSC			Gd – DSC		
	Ischemia	**Unaffected**	**Ratio**	**Ischemia**	**Unaffected**	**Ratio**
Slice 1	5,794	6,265	0,92482	15,714	17,697	0,887947
Slice 2	6,371	5,839	1,091111	19,531	19,263	1,013913
Slice 3	3,502	3,828	0,914838	11,661	12,837	0,90839
Slice 4	3,266	4,667	0,699807	11,414	18	0,634111
Slice 5	4,534	5,396	0,840252	13,425	16,963	0,791428
R^2	0,9633					

Table 9 Representation of the ratio of CBV.

Cerebral Blood Flow:

	N_2 - DSC			Gd - DSC		
	Ischemia	**Unaffected**	**Ratio**	**Ischemia**	**Unaffected**	**Ratio**
Slice 1	113,576	130,337	0,871403	218,336	269,19	0,811085
Slice 2	131,168	174,738	0,750655	207,059	275,349	0,751987
Slice 3	100,48	170,632	0,58887	182,797	285,767	0,639671
Slice 4	100,48	159,424	0,630269	181,532	259,835	0,698643
Slice 5	103,464	153,149	0,675577	183,072	266,085	0,688021
R^2	0,9463					

Table 10 Representation of the ratios of CBF.

Time to Peak:

	N_2 - DSC			Gd - DSC		
	Ischemia	**Unaffected**	**Ratio**	**Ischemia**	**Unaffected**	**Ratio**
Slice 1	25,424	26,878	0,945904	8,442	8,573	0,984719
Slice 2	25,72	24,419	1,053278	8,379	7,322	1,144359
Slice 3	24,121	23,186	1,040326	8,371	7,718	1,084607
Slice 4	26,333	23,721	1,110113	8,191	7,086	1,155941
Slice 5	26,171	22,056	1,186571	7,632	6,169	1,237154
R^2	0,95					

Table 11 Representation of the ratios of TTP.

Mean Transit Time:

	N_2 - DSC			Gd - DSC		
	Ischemia	**Unaffected**	**Ratio**	**Ischemia**	**Unaffected**	**Ratio**
Slice 1	2,367	2,195	1,07836	2,67	2,815	0,94849
Slice 2	2,371	2,106	1,125831	2,713	2,585	1,049516
Slice 3	2,402	2,063	1,164324	2,656	2,408	1,10299
Slice 4	2,302	2,053	1,121286	2,299	2,2	1,045
Slice 5	2,407	2,07	1,162802	2,586	2,2	1,175455
R^2	0,8959					

Table 12 Representation of the ratios of MTT.

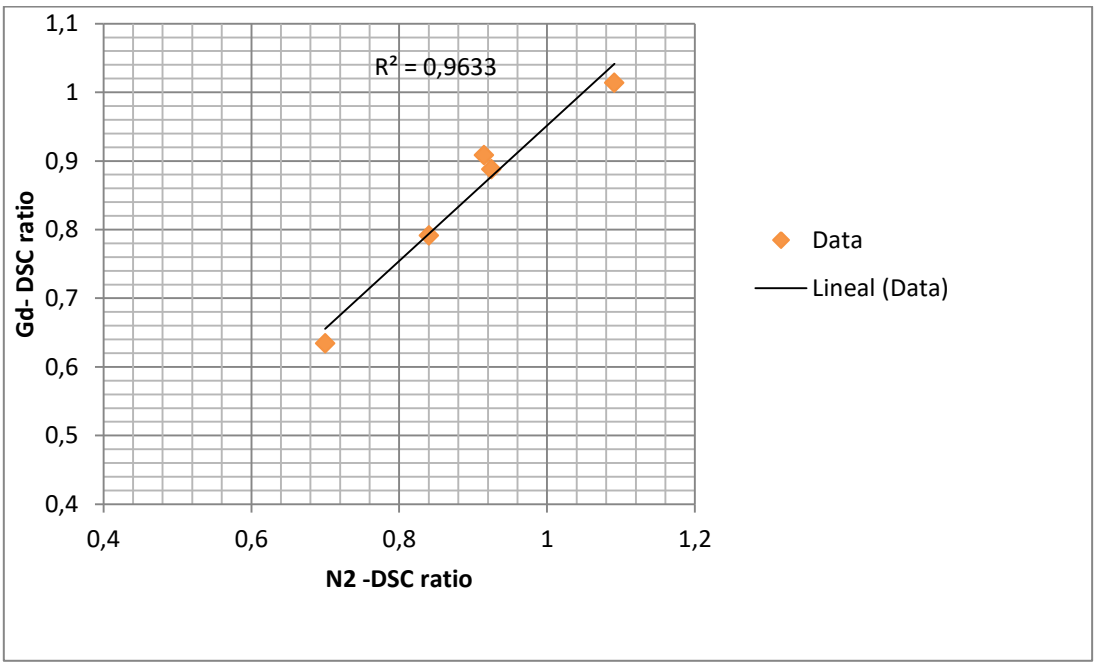


Figure 44 Regression line of Cerebral Blood Volume.

6. Discussion

6.1. Pulse oximeter characterization

The pulse-oximeter curve was studied to determine how certain variables may affect its shape with the intention of obtaining a curve similar to that of gadolinium contrast (ideally an impulse function). Furthermore, the saturation change has to be large enough so as not to be masked by noise. For these reasons, the curve must have a low minimum SpO_2 and be as narrow as possible.

The first variable to be examined was the duration of N_2 breathing period. Three different values were studied: 20, 25 and 30 seconds (Table 6). We realized that the more time the animal spent breathing Nitrogen the more intense the decrease of saturation was. However, it also increased the total width of the curve. In the end, 25 seconds was the time heuristically chosen, as it gives a noticeable enough change in saturation.

The next step was to study the influence of the gas flow in the behavior of the pulse-oximeter curve. Experts do recommend a flow of around 400 ml/min when the animal is connected to the mouthpiece. However, using that flow the saturation of the rodent almost did not change. One of the reasons why this could happen is that the slow flow through the tubes led to the nitrogen getting mixed with oxygen along the way. Another problem is that the mouthpiece where the animal is breathing is not hermetic and air form the surroundings also interfere. The flows studied in the experiment were: 500 ml/min, 600 ml/min and 1 l/min. As it can be observed in Table 7 the flow 1 l/min lowered considerably the saturation, much more than the other flows. Therefore, this was the flow value chosen since the change in saturation is large enough. Another remarkable issue is that as flow increases, the gas arrives faster to the rodent.

Finally, two different lengths of anesthesia tubes were tested (Table 8). The longest tube was the one that matched the MRI scanner setting while the shortest tube was too short to reach the animal inside the scanner. No change was observed in either height or width of the curve using both tubes. The only difference was that the minimum saturation was reached later in the longer tube as expected. These results made no necessary to look for alternative approaches.

Consequently, the parameters chosen were 25 seconds for the duration of nitrogen with a flow of 1 l/ min. As the length of the tube did not affect the results the usual tube setting was kept the experiment.

6.2. Analysis of the signal change during DSC

One of the objectives of the project was determining the relation between the change in saturation and the change in MRI signal. To study this relation, a region of interest that surrounded the whole brain of the animal was drawn with the aid of ImageJ on the images (Figure 31) and the time curve of this region of interest was measured. It is represented simultaneously with the pulse-oximeter curve in the Figure 32. As it can be observed in the figure, the MRI signal has some delay, which is the time the blood takes to move from main arteries to brain capillaries.

N_2-DSC experiment was compared with Gd-DSC (Figure 33) experiment to observe how similar the behavior of both contrasts was, using the same Echo Planar sequence and rodent position. The region of interest selected (Figure 31) in both images and corresponded to the whole brain. It can be seen that in both experiments start to change the MRI signal changes at the same time –few seconds after the introduction of the contrast. However, some differences can be observed between the endogenous and gadolinium contrast (Table 13). One important difference is that the change in saturation is larger in N_2-DSC than in Gd-DSC. Quantitatively speaking, the signal in Gd-DSC decreases a 50% of the baseline signal, while the reduction of N_2-DSC is just about 15%. This is a disadvantage for N_2-DSC as it leads to a lower signal to noise ratio. Another differences between both contrast is that the width of the N_2-DSC $Sp0_2$ is larger, lasting around 25 seconds, while the saturation curve of gadolinium is narrower. However, this should not affect the resulting parametric maps, as the influence of the Arterial input Function is eliminated by the deconvolution procedure.

On the other hand, the endogenous contrast agent has several advantages versus the gadolinium-based contrast. N_2-DSC experiment has a perfect single pass of the contrast while for gadolinium experiment does not immediately recover the initial MRI signal after the pass of the contrast due to recirculation, as gadolinium is not totally removed by the kidneys. This is one important source of error in gadolinium experiments which is solved by using the proposed method. Another advantage of N_2-DSC is that the Arterial Input Function is given by the pulse-oximeter curve; therefore, the AIF is less noisy than that of gadolinium contrast, which is extracted from the image. The level of noise in the Arterial Input Function is a crucial factor, as the deconvolution step for computing the perfusion parameters is very sensitive to noise. The most important advantage of using N_2-DSC over gadolinium is that it is a completed non-invasive procedure with no harmful effects for the patient.

	N_2 - DSC	Gd - DSC
Difference in MR signal	15%	50%
Signal to Noise Ratio	Low	High
Width	25-30 s	10 s
Recirculation of tracer	No	Yes
Arterial Input Function	Pulse-oximeter	Image
Invasive	No	Yes

Table 13 Differences between MRI signal curves of N_2-DSC and Gd-DSC.

6.3. Analysis of signal change in ischemic and unaffected regions of the brain

The Dynamic Susceptibility curves of both the endogenous contrast and gadolinium were calculated in the left part of the brain (ischemic region) and in the right region after stroke-induction (Figure 36 and Figure 37).

The main differences between both regions are that the change in saturation signal is smaller in the ischemic region and that more time is needed to recover the initial values. Both regions of the brain show different behavior, as the parametric maps of the perfusion parameters are computed out of these curves, they will also be different in the ischemic and unaffected region.

6.4. Parametric maps

The parametric maps obtained show a qualitative difference between the rodent before the stroke surgery and after. As it can be observed in section 5.3, the stroke affects mainly the left hemisphere of the brain and, in section 5.4; it can be observe how differently the MRI signal of the DSC sequences change over time. The parametric maps show a difference between the healthy rat and the stroke induced animal, especially in the fourth and fifth slice.

The CBV (Figure 39) shows different values between gray and white matter. Some difference between the healthy and the affected tissue can also be observed, as there is a decrease of volume in the left part of the brain. Table 9 shows a decrease of 15 % - 30%

in slices 5 and 4 in the ratio of N_2-DSC experiments between healthy and ischemic tissue.

The CBF do also vary between gray and white matter regions. Furthermore, it can be observed an important difference between the affected and the healthy region (Figure 40). CBF is a direct measure of the viability of the tissue, as it measures the rate of oxygen and nutrients delivery. Table 10 shows a decrease of about 30 to 40% in the CBF ratio of CBF healthy and ischemic tissues. Figure 41 represents the parametric map of the MTT. MTT shows more homogeneous values than CBV and CBF maps. In the stroke-induced image, we can see that the MTT is prolonged in the ischemic region. This decrease is naturally carried out by the brain in regions that are suffering from low perfusion as a mechanism to increase the time at which the red blood cells are in contact with the permeable walls of the capillaries, allowing more time for oxygen exchange. However, this mechanism is not enough to prevent cells from undergoing ischemia. In Table 12, it can be observed that the Mean transit Time increases around a 15 % in affected regions of the brain.

The last perfusion parameter that was computed is the TTP (Table 11), which is the representation of the time at which the MRI signal reaches the minimum value. In this case, it was measured since the introduction of the contrast. It can be seen that the TTP increases in certain regions of the affected tissue, there is around an 18% increment in the slices most affected by ischemia with respect to healthy tissue.

6.5. Validation of the technique

The use of FiO_2 was validated by comparing this method with Gd-DSC. The gadolinium experiments were performed right after the N_2-DSC, therefore, the slice geometry was kept constant. Gadolinium results were post-processed using the same software used for N_2-DSC experiments. However, in this case the arterial input function was not available. For simplicity, the Arterial Input Function was manually selected from the images, as retrieving the AIF for gadolinium experiment was not the objective of the project and this process is time consuming.

In order to compare the results, the values of the perfusion parameters could not be directly compared as different results were obtained for the two techniques. One of the reasons for having obtained distinct values might be the fact that the Arterial Input Function was not retrieved using the same procedure. Furthermore, the kinetics and metabolic mechanism of the two contrasts is not the same. For example, TTP is shorter in Gd-DSC, since the change using gadolinium occurs at a faster rate (Section 5.2).

For this reason, Gd-DSC and N_2-DSC experiments were analyzed by comparing the ratios between the healthy (right side of the brain) and affected region (left side of the brain). This quotient was computed for both methods and compared using linear regression. In section 5.6, it can be observed that the coefficient of determination (R^2) is above 0.9 in CBV, CBF and TTP perfusion parameters, while MTT has a value of 0.89.

Consequently, there is a high correlation between both methods, confirming that N_2-DSC can be utilized as a method for measuring brain perfusion.

7. Conclusions & Future improvements

The project did manage to fulfill the proposed objectives. The scanner was automatically synchronized with the gas-mixer using a MATLAB code which controlled both devices. The synchronization was simple and avoided possible human delays, furthermore making it easier the control of the gas mixer, as the user one just needs to press one key to activate the whole program.

The pulse-oximeter was carefully characterized and a narrow curve was finally obtained. The change in MRI signal is not as noticeable and narrow as in gadolinium experiments; however, the results were good enough to detect the change and compute the parametric maps, without the prejudicial effects of an invasive contrast. Furthermore, the parametric maps obtained for gadolinium and changes in FiO_2 are comparable and have similar behavior, as it was studied in section 5.6. The parametric maps were computed using a MATLAB code that was complemented with a GUI interface which allows the user to introduce the data and visualize the results in a faster and easier way.

7.1. Future improvements

There are several improvements which could be implemented in order to improve the efficacy and accuracy of this project. Furthermore, there exists the possibility of expanding the project to other organs of interest.

One of the possible improvements would be to optimize and sophisticate the code involved in image post-processing. Developing a better masking of the brain for getting rid of the background noise could be a possible improvement, as currently the mask is computed by doing a simple threshold, more developed methods, such as Otsu's segmentation could be implemented. Another possible change may be performing a more advanced method for deconvolution of the Arterial Input Function to obtain the Mean Transit Time, since the current method (Singular Value Decomposition) is very sensitive to noise and there are certain pixels that did not provide meaningful values.

Another thing that could be done in the future to study N_2-DSC is examining the behavior of perfusion in rodents with a brain tumor. This was not addressed in the project, as it is easier to induce a stroke. However, the assessment of perfusion in this kind of subjects could be interesting as clinical DSC perfusion is mainly performed for patients who suffer from brain tumors.

In the future, after having performed several pre-clinical experiments, there exists the possibility of translating the studies to the clinical phase and studying the feasibility of using changes in fraction of inspired oxygen in humans.

On the other hand, this experiment could be expanded to other parts of the body, such as for example the heart. Dynamic Susceptibility Contrast is a method that is only used in brain. This is because the fundamental property of DSC assumes no retention or pooling of the contrast. Due to the presence of the blood brain barrier in the brain, gadolinium brain contrast is not able to traverse the vessels – under normal conditions – and go to the extracellular space. However, this does not occur in other organs of the brain, as they do not possess blood brain barrier, and the contrast is retained for some time in the extracellular space. This would not happen using N_2-DSC, as hemoglobin is always inside the vessels and it does get trapped in the extracellular space. It could be an innovative way to study blood perfusion in different organs of the body.

8. References

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APPENDIX A

MATLAB code for synchronization of hardware

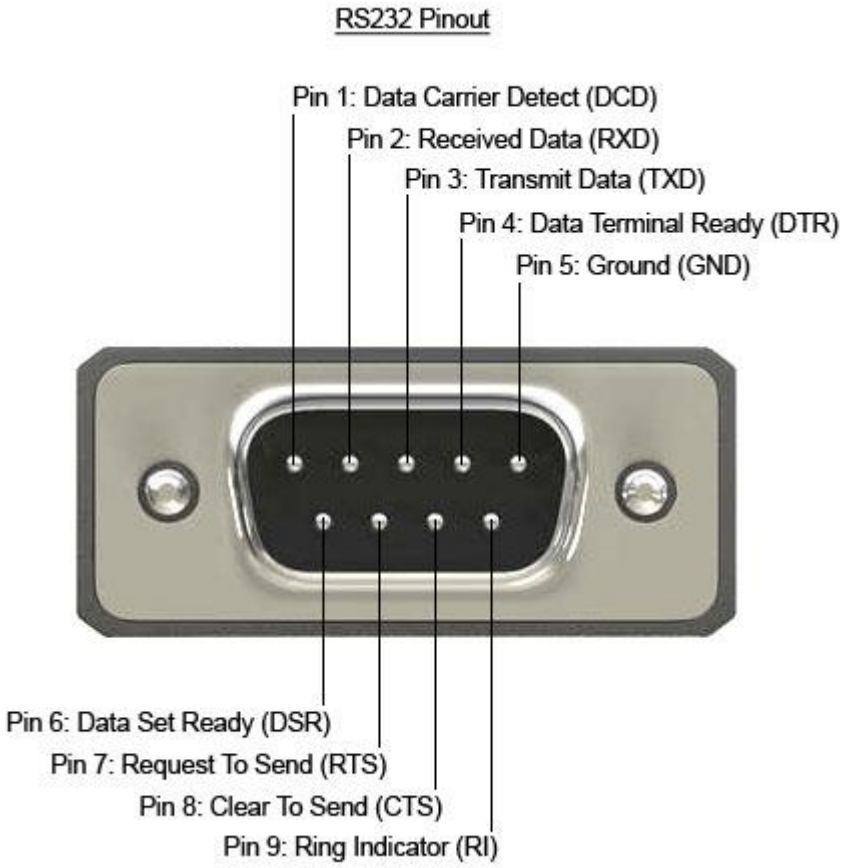
```
%Create object from Port Gas Mixer
obj1 = instrfind('Type', 'serial', 'Port', 'COM3', 'Tag', '');
if isempty(obj1)
    obj1 = serial('COM3');
else
    fclose(obj1);
    obj1 = obj1(1);
end
%Set the required baudrate and databits
set(obj1,'BaudRate',19200);
set(obj1,'DataBits',8);
%Open Port Gas Mixer
fopen(obj1);
%Select the first mixture: 100% O2 0% N2
fwrite(obj1,'1')

%Create object from Port Scan
obj2 = instrfind('Type', 'serial', 'Port', 'COM4', 'Tag', '');
if isempty(obj2)
    obj2 = serial('COM4');
else
    fclose(obj2);
    obj2 = obj2(1);
end

%Open Scan Port
fopen(obj2);

t1 = clock;
%Read continuously Ring Indicator from Scan Port, when b is
'off' exit the
%loop
b = obj2.Pinstatus.RingIndicator;
while isequal(b,'on')
  clear b
  b = obj2.Pinstatus.RingIndicator;
end
pause on
t2 = clock;
pause(30)
t3 = clock;
% Send the mixture 0% O2 100% N2 to the gas mixer
fwrite(obj1,'2')
pause(25)
% Send the mixture 100% O2 0% N2 to the gas mixer
fwrite(obj1,'1')
%Record the time
Tepi = [t1, t2, t3];
```

APPENDIX B



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APPENDIX C

Graphs for pulse-oximeter characterization

- Duration of 100% N_2 stage:

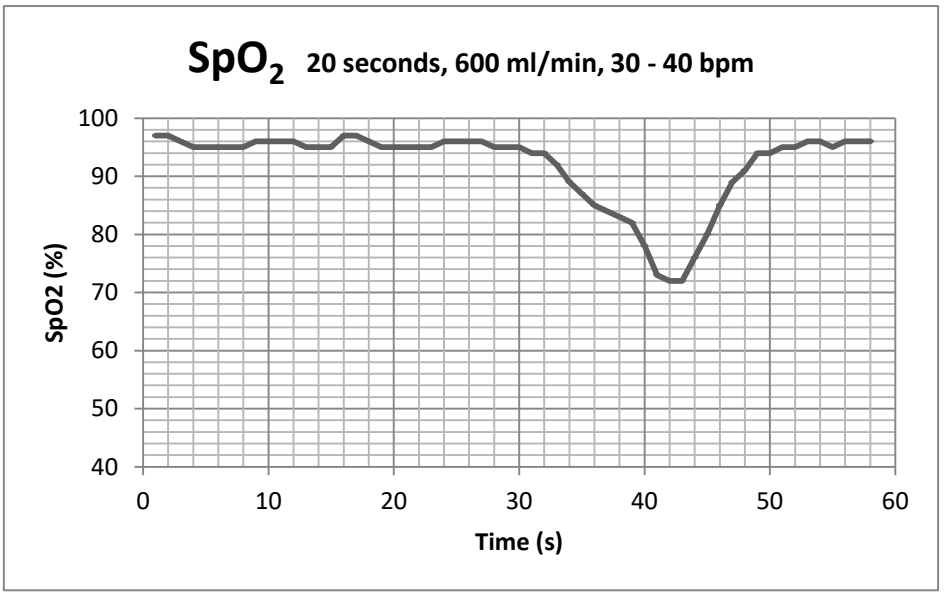

Figure C.1 SpO_2 curve of 20 seconds of Nitrogen.

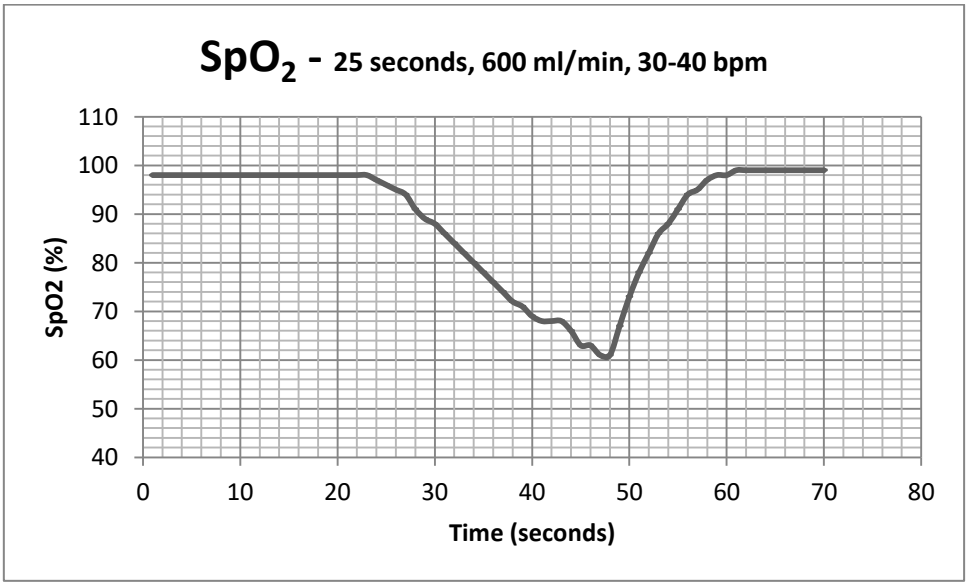

Figure C.2 SpO_2 curve of 25 seconds of Nitrogen.

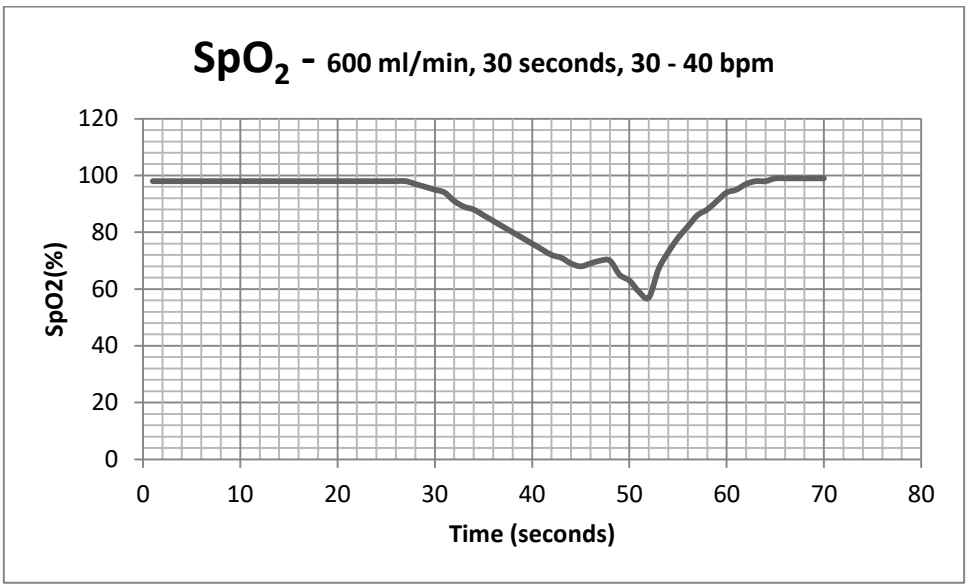

Figure C.3 SpO_2 curve of 30 seconds of Nitrogen.

- Gas mixture flow:

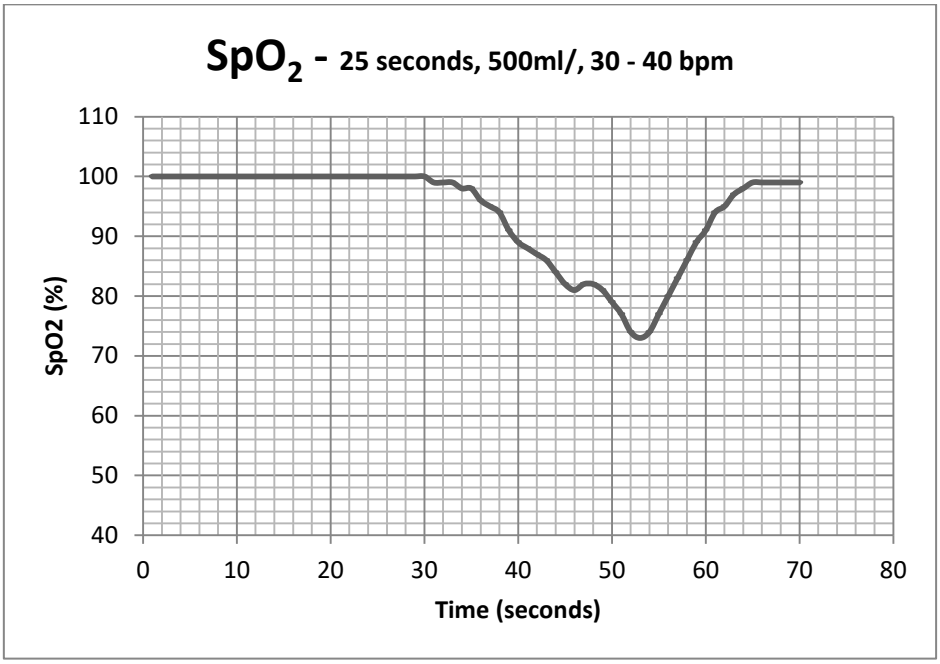

Figure C.4 SpO_2 curve using 500 ml/min of gas flow.

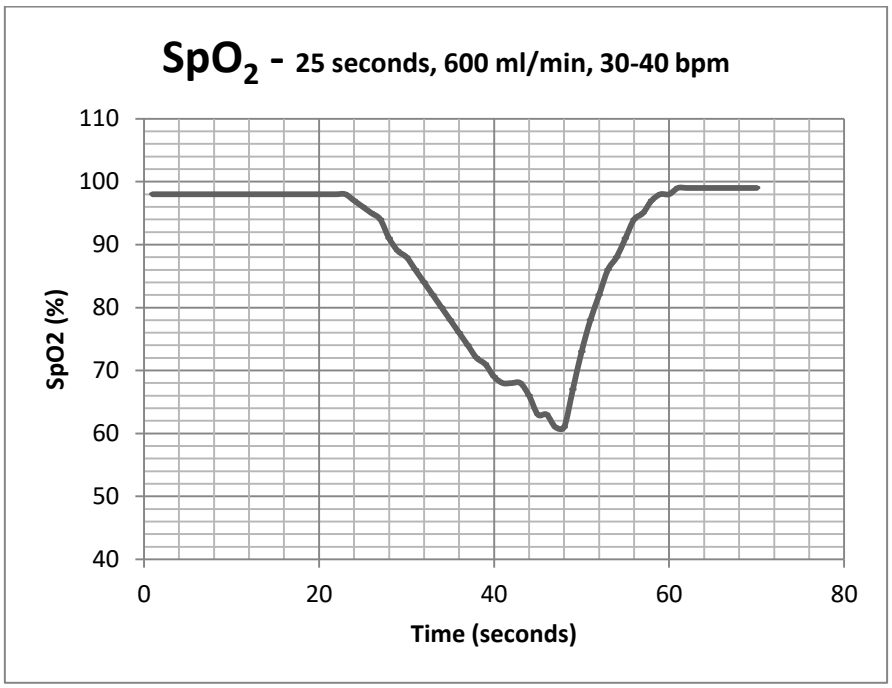

Figure C.5 SpO_2 curve using 600 ml/min of gas flow.

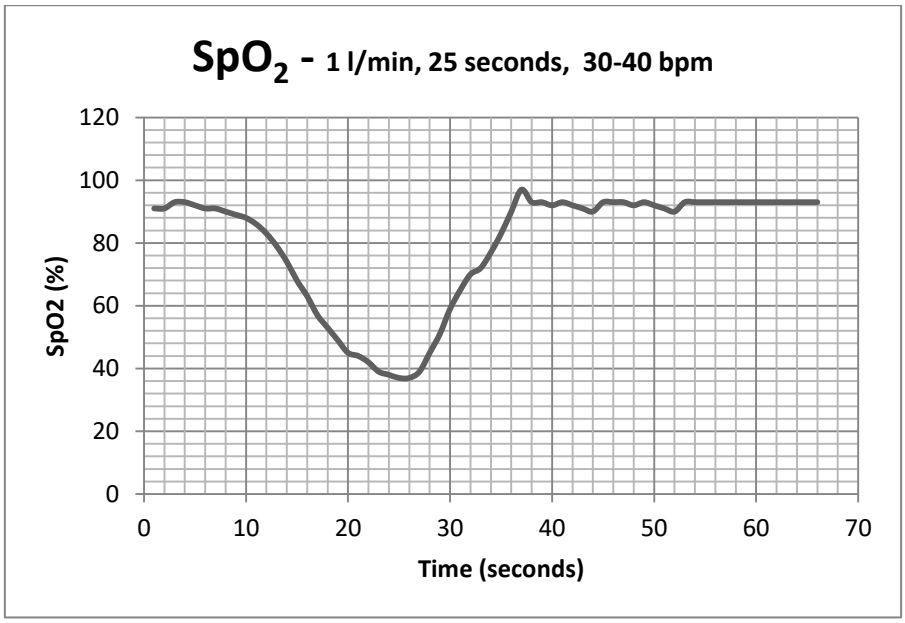

Figure C.6 SpO_2 curve using 1000 ml/min of gas flow.

- Length of gas tubes:

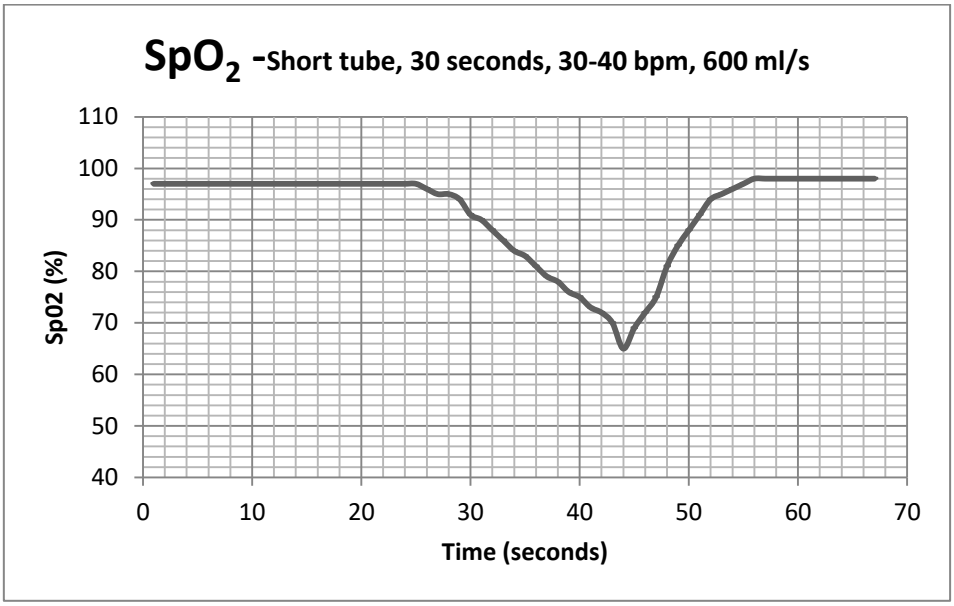

Figure C.7 SpO_2 curve using a shorter tube.

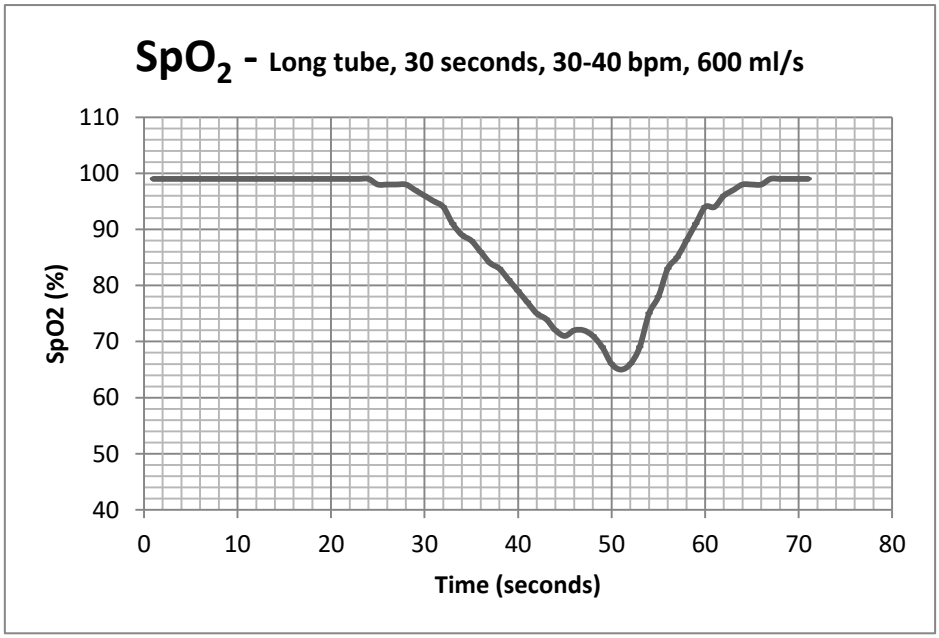

Figure C.8 SpO_2 curve using the usual tube.